An Institute of Microbiology—Its Aims and Purposes

Selman A. Waksman

Rutgers University, New Brunswick, New Jersey

MICROBIOLOGY comprises the field of knowledge of various microscopic forms of life including the bacteria, fungi, yeasts, actinomycetes, protozoa, and algae. This science embraces a study of the occurrence of microorganisms in nature, their nutrition, their structure and life cycle, their role in numerous natural processes, and their relation to man, to domesticated and wild animals and plants, and to one another.

Although various microbes have been utilized by man since time immemorial and although some of the diseases caused by microbes have been combated since prehistoric man, the science of microbiology is of very recent origin. One of the youngest of sciences, it was hardly recognized as such a hundred years ago. Particularly striking progress has been made in late years, however, in our knowledge of the fundamental aspects of the physiology and nutrition of microorganisms and in the practical applications of this knowledge. In a relatively brief time, an important field of science has come into being—a science with applications in virtually every field of human endeavor. Microorganisms are recognized now as playing very important roles in natural processes; they are the most important causative agents of human, animal, and plant disease; they are the cause of fermentation and therefore play an important part in certain industrial processes and in the preparation of many foodstuffs and beverages; they are largely responsible for food spoilage, deterioration, and destruction of all types of materials, ranging from textiles to steel pipes; and finally they are recognized as the all-important agents in a great number of soil processes. More recently, with the development of our knowledge of the production of antibiotics and their use for combating a wide variety of diseases, microorganisms have come to occupy another highly important role in human economy.

Unfortunately, microbiology was heretofore treated as an inferior among the older sciences. It was usually considered a handmaiden of various applied sciences and, as such, was accorded a second place. It was always subservient to such fields as medicine, where it was closely tied up with pathology; public health, where it was associated with sewage disposal and sanitary conditions in general; and agriculture, where it was concerned largely with soil processes, composts, and the preparation of silage and foodstuffs.

Microbiology, it may be said, has not yet come into its own. This may be due partly to the fact that the two greatest early bacteriologists were interested not in microbiology as a fundamental science, but rather in its applications. Louis Pasteur was a chemist and Robert Koch was a clinician. They were more concerned with brewery and fermentation problems on the one hand, and with infectious diseases on the other, than with the organisms causing these. It is true that Ferdinand Cohn, a botanist, fully appreciated the significance of bacteria as biological systems, but neither he nor the botanists following him recognized the great importance of this observation; they allowed the medical people and the fermentation industries to take over microbiology and, primarily, its application. Microbes were thus considered as agents responsible for certain diseases or for certain important natural processes; they were to be either discouraged or encouraged, whichever the case might be, but they were to receive only scant recognition as independent biological systems.

It is further important to note that the general tendency has been, until recently, to limit the subject of microbiology largely to the study and utilization of bacteria. It was, for example, only four or five years ago that a group of botanists came to the conclusion that microbiology is a field of knowledge in itself. They then proceeded to recognize it by organizing a new society of microbiology—apparently in order to correct the blunder made by the botanical world some seventy-five years ago, in failing to recognize the great potential importance of the bacteria and other microscopic forms of life studied by Ferdinand Cohn.

On the other hand, many bacteriologists have also erred in this direction, some going so far as to consider the disease-producing bacteria as the only microscopic forms of life worth studying. The very large number of saprophytic microorganisms which comprise bacteria, fungi, and actinomycetes, were usually either dismissed from consideration entirely or referred to casually as organisms that belong to the domain of soil bacteriology, and have, therefore, limited importance. One illustration will suffice. William Bullock, an outstanding British bacteriologist,

who has contributed to the science of microbiology one of the most comprehensive histories of the subject, not only omitted consideration of the fungi and the actinomycetes, but even among the bacteria he gave little or no attention to the autotrophic and to the nitrogen-fixing organisms—two groups of bacteria that occupy a unique place in microbiology because of their specific physiological properties, their significance in bacterial taxonomy, and their great economic importance.

Because of such neglect, even on the part of experts in the field, many microbiologists felt rather skeptical about proper recognition of their branch of science. In addressing the Third International Congress of Microbiology on the eve of World War II, Prof. Kluyver, one of the leading microbiologists in the world today, spoke bitterly of the lack of recognition of microbiology as a field of science. He even anticipated attacks upon his lecture, and said that some critic might write: "The lecture gave additional evidence of the correctness of my earlier statement that it is questionable whether bacteriology is entitled to the rank of science. And if some benevolent judge might be willing to answer this question in the affirmative sense, then it should be remarked that the lecture showed once more that bacteriology is a science which has no laws and almost no bacteriologists."

Kluyver was not the only microbiologist who voiced bitter disappointment in the prevailing attitude toward this science. By way of another illustration, I cite a statement made privately to me by another prominent microbiologist, Charles Thom, formerly of the United States Department of Agriculture, to the effect that a colleague of his once remarked that soil microbiology had been *the most futile field of labor*—judged by what it produced—that he had any knowledge about.

It is not my purpose either to defend the science of microbiology or to magnify its great theoretical and practical importance. I only emphasize the fact that during the last few years microbiology has had focused upon it the attention of both the scientist and the practical man. I should like to give consideration here to the importance of only two groups of microorganisms—namely, the fungi and the actinomycetes, both of which previously were largely neglected, except in certain limited fields of application.

At no time in past history have these two groups of microorganisms played so great a part in the affairs of man as during World War II. Few laymen, even among those who participated in the war, appreciated the great importance of the fungi—as agents of destruction of essential materials, as causative agents of human diseases, and as producers of drugs, such as penicillin, that could be utilized successfully for combating many of the diseases caused by bacteria. Because of these important activities, the fungi tended to overshadow even the bacteria and other microbes, notably the protozoa and the viruses, in spite of the fact that these continued their important role in human economy, especially in the causation of disease and epidemics.

It is true that fungi have long been recognized as having great potentialities for destroying a great variety of materials. This was appreciated particularly by the plant pathologist, who was continuously faced with the fact that fungi are more concerned in the diseases of cultivated and wild plants and cause greater damage than all other organisms combined, with the possible exception of insects. The soil microbiologist, too has long known that fungi are responsible for the major destruction of the plant and animal residues in composts and in soil, and thus not only contribute to the removal of wastes, but also lead to the liberation in available form of the chemical elements essential for plant growth. And fungi are no strangers to the housewife, who in her attempt to preserve jams, jellies, bread, and other foodstuffs, is continuously faced with the problem of combating spoilage by a great variety of molds. The lover of mushrooms also appreciates the fungi, but as a friend rather than as an enemy. The great potentialities of fungi have also been recognized in certain other fields of human endeavor. It is sufficient to mention the manufacturer of foods, such as cheeses; of beverages such as beer and distilled liquors; and of industrial products, such as citric acid, gluconic acid, fumaric acid, ethyl alcohol, and glycerol.

As already mentioned, it was only during the last war that full recognition was given to the great importance of fungi. With the concentration of large quantities of service materials in tropical and subtropical areas under the prevailing conditions of high humidity and high temperature, the damage potential of the fungi became apparent. Fungi attacked optical and electrical equipment, clothing, tents, and other materials used by service personnel, and even infected the human body. Although the physiology of the fungi and methods of their control had received much consideration, especially by plant pathologists and soil microbiologists, discoveries in these fields did not lend themselves readily for application to the problem at hand. Thus a rapid survey had to be made of the nature of the fungi causing the damage, the added role of bacteria, insects, mites, and other lower forms of life, the nature of the damage brought about, the nature of the fungistatic and fungicidal agents required to protect different kinds of service materials, the methods for measuring the protection thus rendered, and a host of other problems. It was only

natural that all these problems, dealt with under pressure of wartime conditions, were studied largely from the point of view of immediate necessity and not from that of a fundamental science. Here was a biological problem that should have been investigated in great detail, so that information would have been ready for immediate use. It required the coordinated efforts of many investigators, and resulted in great accomplishments, none greater than the emphasis laid upon the importance of fungi in natural processes.

Antibiotics were another wartime development. Just on the eve of World War II, I said in a public address: "We are finally approaching a new field of domestication of microorganisms for combating the microbial enemies of man and of his domesticated plants and animals. Surely microbiology is entering a new phase of development. This new phase has now been called the subject of antibiotics."

Just what are antibiotics? This question was placed before me less than eight years ago, in 1941. The following definition is based upon my earlier designation of the term: "An antibiotic or an antibiotic substance is a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other microorganisms." The action of an antibiotic against bacteria and other microorganisms, as distinct from common antiseptics and disinfectants, is selective in nature, since only some bacteria are affected by a given agent, and others not at all, or only to a limited extent. Antibiotics vary greatly in their toxicity to animals and in their activity *in vivo*. Because of these characteristics, only a very few of the antibiotics so far isolated have received recognition as chemotherapeutic agents.

I need hardly dwell upon the great progress made in the field of antibiotics during the last seven or eight years. Although the chemist feels somewhat slighted because the clinician is claiming most of the credit for developing this field, and although the pharmacologist and physiologist have made their important contributions, you will agree with me that it is the microbiologist who is entitled to the credit for having opened up this field of science and for continuing to make substantial contributions to its development.

What has been said of the importance of fungi applies to a greater extent, though in a somewhat different way, to the actinomycetes. Here is a large and heterogeneous group of microorganisms which had aroused little interest on the part of the microbiologists or any other group of investigators. An important plant disease or two, a rare and peculiar human or animal infection encountered at very infrequent intervals, abundant occurrence in the soil—this much was known about the actinomycetes—but nobody cared to waste much time in determining what these delicate, mycelium-producing, odoriferous, microscopic forms of life do either in the soil or in a manure pile. It is the field of antibiotics that has focused attention upon the possibility of utilizing certain hidden physiological properties of these organisms. When one realizes that twenty percent or more of all actinomycetes that are freshly isolated from natural substrates have been found capable of inhibiting the growth of bacteria or other microorganisms, one comes to appreciate their great potentialities in this respect.

Thus our recently developed knowledge of vitamins and their role in human nutrition, of the utilization of penicillin, streptomycin, aureomycin, chloromycetin, and other antibiotics in the control of disease, of the potential part that bacteria may play as agents of biological warfare, of the destruction of service materials under tropical conditions, of industrial fermentations, and of many other microbiological processes has focused renewed attention upon the vast number of applications of microorganisms and their great importance to our economy, public health, and medicine. It is, therefore, essential to recognize these developments and to plan a comprehensive study of the fundamental aspects of the nature and activities of various groups of microorganisms, their role in natural processes, and their use in agriculture, industry, public health, and other aspects of human economy.

With this in view, an Institute of Microbiology is being established by Rutgers University. In this institute particular attention will be devoted to the fundamental aspects of the study of microorganisms, their physiology, their biochemical activities, and their relations to higher forms of life, notably to man and to his domesticated animals and plants. In other words, microbiology will be treated as a fundamental science. Certain of the practical or applied phases resulting from these studies will be carefully examined by other divisions of the institute.

In planning the program of the Microbiological Institute, the broad field of microbiology is conceived as covering six of the major groups of microorganisms, namely bacteria, actinomycetes, filamentous fungi or molds, yeasts, protozoa, and viruses. Minor consideration may also be given to other groups, such as the higher or mushroom fungi, the algae, certain worms, and other microscopic forms of life.

The functions of the institute will comprise both research and teaching, largely on graduate student and postdoctorate levels. The staff of the institute will offer courses in the various fields of microbiology to graduate students at Rutgers University and will conduct seminars for graduate and postdoctorate stu-

dents. These courses will also include supervision of various research problems, in both the fundamental and the applied phases of the subject. The institute will serve as a gathering place for seminars and conferences on microbiological subjects. It will also serve as a depository of cultures of microorganisms of theoretical or practical importance.

Although it is not intended to overorganize or departmentalize the Institute of Microbiology, it is tentatively proposed that it should include six major divisions or fields of research:

I. *General Microbiology.* This division will be concerned with the study of the structure and functions of microorganisms. Particular attention will be paid to morphology, cytology, genetic characteristics, taxonomic relationships, and ecology.

II. *Microbial Physiology.* This division will be concerned with the study of the physiology of different groups of microorganisms, their nutrition, intermediary metabolism, and other biochemical properties. Its program will include studies of the physiology of certain representative types of organisms found among the pathogenic and saprophytic bacteria, fungi, actinomycetes, viruses, and others.

III. *Antibiotics of Microorganisms.* This division will be concerned with the study of the formation of antibiotics by microorganisms, their isolation and purification, their chemical composition, their mode of action upon bacteria and other microorganisms, and their utilization for disease control. Particular attention will be paid to the isolation of antibiotics active against *Mycobacterium tuberculosis,* against viruses, and possibly against tumor cells also.

IV. *Vitamins and Enzymes.* This division will be concerned primarily with the study of the role of vitamins and enzymes in the nutrition of microorganisms, their production under different conditions of growth, and their utilization.

V. *Ecology of Microorganisms.* This division will be concerned with the occurrence, activities, importance, and control of microorganisms in soils, composts and water basins, in various agricultural products, and in foodstuffs.

VI. *Applied Microbiology.* This division will deal primarily with the application of microorganisms. Problems given consideration here will be largely of a practical nature. It is sufficient to mention such fields as the microbiology of foodstuffs, microbiology of soils and manures, microbial fermentations, causation of spoilage and methods of control, antiseptics and disinfectants, and finally—and not least important—the relations and interrelations of microorganisms in human, animal, and plant diseases.

Although the highly important fields of medicine, veterinary science, and various concomitant branches, such as immunology and disease control, will be avoided in principle, certain aspects will be considered in connection with some of the work proposed for the institute whenever the problems under consideration necessitate the study of disease-producing organisms, their physiology, and methods of control. This may involve collaboration with pharmacologists and medical investigators in industrial laboratories and in medical institutes.

Surely microbiology may be said to have come of age. Surely the establishment of an institute which is to be devoted to the task of training advanced students in the field of microbiology and to planning a program of research in the fundamentals of microbiology needs no apology. It is a great field, rich in scientific potential and in practical applications. The future for this field is bright indeed.

Based on an address delivered May 3, before representatives of the press and radio, on the organization of the Institute of Microbiology at Rutgers University, the State University of New Jersey.

On the Interpretation of the Absorption of Ultraviolet Light by Cellular Nucleic Acids

Barry Commoner

The Henry Shaw School of Botany, Washington University, Saint Louis, Missouri

EFFORTS TO EXTEND the field of quantitative biochemistry to the level of the single cell have been based largely on absorption spectrophotometry. This method has suggested a means of determining intracellular concentrations of specific substances by virtue of the proportional relationship between the number of molecules in the light path and the extinction at the wavelength characteristically absorbed by the compound in question. ($E = \log I_0/I = \varepsilon cd$).[1] This relationship, the Beer-Lambert law, usually holds for the absorption of light by gases and solutions, and—for the purpose of determining intracellular concentrations—is *assumed* to be true for the physical states encountered in living and dead cells (*8, 17, 18, 32, 33*). Conclusions based on this assumption have led to the development of a number of theories concerning the biochemistry of the nucleus, nucleic acid metabolism, protein synthesis, and metabolism of tumor cells (*8*).

However, it has been recently suggested (*12*) that the amount of light specifically absorbed by the substance under study may vary considerably with the degree of orientation imposed on the material. It was shown that when the following two conditions exist together, materials will fail to absorb more than one-half the incident light and thereby depart widely from the Beer-Lambert law: (a) the molecules absorb light by virtue of a distinctive electronic axis (i.e., one occupying a unique position in the structure of the molecule) and (b) the molecules exist in an oriented aggregate.

Most intracellular extinction measurements have been intended as determinations of intracellular nucleic acid content, and these data have figured largely in the development of the theories cited above. It would seem important, therefore, to determine to what degree the two conditions required for anomalous absorption apply to the nucleic acids occurring in cellular structures. As will be shown below, such an analysis suggests that the extinctions of intracellular objects at 260 mμ do not necessarily reflect their actual nucleic acid content, and that considerable caution must be used in interpreting such data.

[1] E = extinction, I_0 = intensity of incident light beam, I = intensity of transmitted beam, c = concentration of absorbing material in moles/liter, d = thickness of the sample in cm, and ε = molar extinction coefficient.

Optical properties of nucleic acids.

The characteristic ultraviolet absorption of the nucleic acids is a property of their constituent purine and pyrimidine bases. According to the theory of color developed by Lewis and Calvin (*21*), the absorption of light is due to specific electronic oscillations within the structure of a molecule. Hence, for planar molecules such as the purines and pyrimidines, these oscillations can be resolved into lines in this plane.[2] As shown by Lewis and Calvin and by Ferguson (*13*), molecules which are nonsymmetrical with respect to the position of the groups responsible for their color have two fundamental electronic axes, both lying in the plane of the molecule. These axes are characterized by different wavelengths, the oscillation of lower energy representing the absorption maximum at the longer wavelength.

In some cases Lewis and his collaborators (see reference *12* for references) have localized these axes with respect to the dimensions of the molecule by determining the effect of the plane of polarization of the incident light on the extinctions yielded by molecules in rigid media. They have shown for such cases that the extinctions at the two characteristic wavelengths reach maxima at planes of polarization which are at 90 degrees to each other.

Unfortunately, comparable data for the nitrogen bases are lacking, and in discussing them one is forced to deal with assumptions based on analogy with the molecules studied by Lewis *et al.* The lack of symmetry of the nitrogen bases suggests that they possess two distinctive absorption axes lying in the plane of the molecule. Except for guanine, these substances yield a single maximum in the ultraviolet (see Hotchkiss, *16*) at 260–265 mμ in neutral solution. This maximum must represent either (a) the absorption

[2] Such molecules may have a third axis representing electronic oscillations perpendicular to the plane of the molecule. The energy level of such oscillations is so high that this axis is characterized by light absorption at wavelengths too short to fall within the range involved in ordinary spectrophotometry of solutions. This type of absorption will therefore not be considered.

due to one of the two distinctive axes; or (b) absorption due to both axes.

If the first assumption is the correct one, the maximum at 260–265 mμ must represent one of the axes, and the second axis must account for an absorption maximum at a significantly shorter wavelength. The second maximum would probably lie in the range below 220 mμ and therefore be undetectable by spectrophotometry of solutions. If alternative (b) is the correct one, the axes must be characterized by nearly identical wavelengths. In the absence of critical data, it is of course impossible to choose between these alternatives, but on the basis of what is known about the optical properties of nonsymmetrical molecules, the second alternative seems the less likely one. Hence for the purposes of this discussion we shall assume that the absorption of the nitrogen bases (other than guanine) at 260–265 mμ is due to a distinctive electronic axis which lies in a unique position with respect to the dimensions of the molecule. If this assumption is incorrect, the analysis which follows is altered in certain details, which will be noted below.

The structure of nucleic acids has been discussed by Astbury (*1*) who concludes that the constituent nucleosides are arranged in a parallel stack, so that the entire molecule has the configuration of a roll of coins (where each coin is a nucleoside). He also suggests that each nucleoside is probably oriented in the same way with respect to the long axis of the molecule, thereby placing the rings of the nitrogen bases in complete alignment along the length of the molecule.

It follows, therefore, that the absorption axes of the nitrogen bases which account for the extinction of nucleic acids at 260 mμ must be rigidly oriented throughout the entire polynucleotide. Although the available data are insufficient to describe completely their position with respect to the dimensions of the nucleic acid molecule, these axes must lie in planes perpendicular to the long axis of the molecule. This is in accord with Schmidt's observation (*27*) that the birefringence of thymonucleate fibers is negative with respect to the long axis of the fiber, and the finding of Signer, Caspersson, and Hammersten (*30*) that flow birefringence is negative to the direction of flow. It also agrees with Caspersson's observation (*7*) that for maximum absorption at 257 mμ by partially oriented films of thymonucleic acid, light must be polarized in a plane perpendicular to the long molecular axis.

Similar results have been obtained by Butenandt *et al.* (*3*), who studied the absorption spectrum of flowing solutions of tobacco mosaic virus with plane polarized light. They found that the extinction at 260 mμ was at a maximum when the light was polarized in a plane perpendicular to the direction of flow (and therefore also perpendicular to the long dimension of the molecules oriented by flow), and concluded that the absorbing groups must occupy planes perpendicular to the long axis of the virus nucleic acid (which parallels the long axis of the virus particle itself).

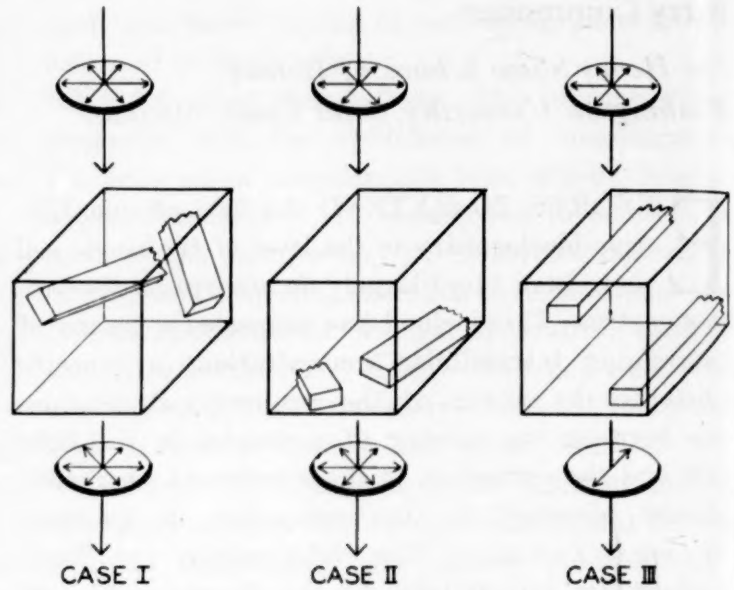

FIG. 1A. The effect of orientation of nucleic acid on the absorption of unpolarized light. The nucleic acid molecule is represented as an elongated oblong prism (only part is shown) whose smallest face is parallel to the planes occupied by the nitrogen bases. In Cases I and II light of all electric vectors is equally transmitted; in Case III only light with a vector perpendicular to the long axis of the molecule is transmitted. Since this represents 50% of the incident light, Case III gives a maximum extinction of 0.3. The dimensions of the molecule are arbitrary.

This visualization of the optical structure of nucleic acid molecules makes possible consideration of the effect of molecular orientation on the extinctions yielded at 260 mμ. Since the preponderance of the constituent nitrogen bases (i.e., except guanine) probably contribute to the absorption by virtue of a distinctive electronic axis, and appear to be rigidly ordered along the length of the polynucleotide, the orientation of these axes is determined by the position of the molecule itself.

Hence we may consider the three cases of orientation of optical axes discussed in reference (*12*) in terms of the possible types of orientation of the nucleic acid molecule proper. These are diagramed in Fig. 1A. In Case I, the molecules, and the axes responsible for the absorption at 260 mμ, are randomly distributed in space. The relation between the number of molecules scanned (N) and the extinction (E) at 260 mμ is therefore a straight line, and the Beer-Lambert law is obeyed. In Case II the orientation is

at random within a series of parallel planes. The relation between E and N is again linear, but not identical with that of Case I. In Case III the molecules, and therefore the absorption axes, are completely oriented in three dimensions. Consequently, the relation of E to N is nonlinear above very low E values, and the system departs radically from the Beer-Lambert law. In this type of orientation, the fraction of incident light absorbed cannot rise above 1/2 and E cannot exceed 0.3. As a result, E becomes independent of the number of molecules scanned as N increases.

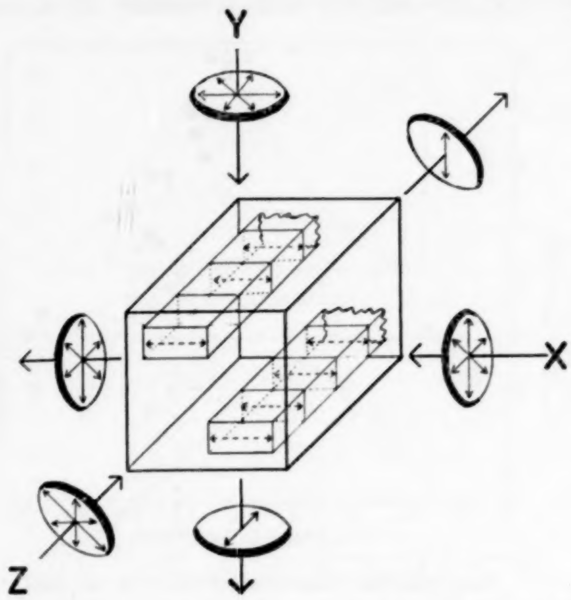

FIG. 1B. The absorption of unpolarized light impinging on an oriented aggregate of nucleic acid molecules from various directions. The nitrogen bases are represented as planes parallel to the front (smallest) face of the molecule; the dotted arrows in these planes represent the position of the distinctive electronic axis responsible for the absorption at 260 mμ. The dimensions of the molecule are arbitrary.

This description pertains to the situation in which the nucleic acid molecules are oriented with their long dimension perpendicular to the direction of the incident light beam. Since this relationship may not always be true of cytological preparations, the properties of oriented aggregates viewed from all possible directions need to be considered.

Fig. 1B describes the optical properties of a fully oriented nucleic acid aggregate when the incident light beam impinges on it from various directions. As shown in Fig. 1A, when the incident beam is perpendicular to the long dimension of the molecule and also to the distinctive absorption axes of the nitrogen bases (i.e., the beam parallels the Y axis), only light with an electric vector parallel to the planes of the nitrogen bases can be absorbed. Thus, the emergent beam is plane polarized and necessarily represents a minimum of 1/2 the energy of the incident beam. The limit of E is therefore 0.3, regardless of the number of molecules in the path of the beam.

The same effect occurs when the incident beam is propagated along the Z axis (parallel to the long dimension of the molecule), for here, too, the beam is perpendicular to the absorption axes. Thus, if the aggregate is examined from the direction of the Y axis, or the Z axis, or from any intermediate direction, the absorption effects typical of Case III occur.

When the incident beam is parallel to the X axis, and is therefore also parallel to the absorption axes of the nitrogen bases, the probability that any photon will be absorbed is zero, and the molecule is transparent (at 260 mμ) from this direction. Consequently E is zero for all N values. For beams impinging from directions between the X and Y axes, and between the X and Z axes, the relation between E and the number of molecules will be as follows: E cannot rise above 0.3, but (with increasing values of N) will approach this limit more slowly that it does when the beam is on the Y or Z axis.

Thus, if (as assumed) the absorption at 260 mμ by an oriented nucleic acid aggregate is determined by a distinctive absorption axis, extinctions greater than 0.3 cannot occur, regardless of the direction from which the aggregate is examined. Differences in the direction of the impinging beam only affect the rate at which this limit is approached with an increase in the number of molecules in the beam's path. This suggests that cytological preparations which contain oriented aggregates of nucleic acid may yield extinction values which do not follow the Beer-Lambert law and therefore are not necessarily proportional to the number of molecules scanned.[3]

The orientation of nucleic acids in cell structures.

While there is considerable information on the orientation of molecular aggregates in cellular structures, decisive data on the orientation of nucleic acids proper are rather sparse and contradictory. Most of the evidence is summarized in the exhaustive reviews of Schmidt (*27*), Frey-Wyssling (*14*), Schmitt (*28*), and Monné (*22*), and unless otherwise indicated, ref-

[3] If the nucleic acid absorption at 260 mμ is *not* due to a distinctive electronic axis, these relationships are altered as follows: (1) Case I and Case II yield identical straight lines. (2) In Case III, the limit of E is 0.3 when the incident beam parallels the Y axis or the X axis or any direction between these two. When the beam impinges along the Z axis, the relation of E to N is linear and the Beer-Lambert law is obeyed. For positions between the X and Z axes, and the Y and Z axes, the relation of E to N is not linear, but approaches the extreme (in which the limit of E is 0.3) as the X and Y axes are approached. Thus, even if it is not assumed that the nucleic acids are characterized by a distinctive absorption axis at 260 mμ, oriented aggregates will not follow the Beer-Lambert law except in the rare case when the molecules are examined from a direction exactly parallel to their long dimension.

erences relative to the discussion which follows may be found in these sources.

That preparations of extracted nucleic acids may form fully oriented aggregates is evident from Caspersson's observations (*7*) of dichroism in thymonucleate gels. Such aggregates are also birefringent, the sign of this effect being negative with respect to the long axis of the aggregated molecules. This provides a method of distinguishing birefringence due to nucleic acids from that due to aggregates of parallel protein chains, for the sign of the latter is positive with respect to the long axis.

Practically all cytological structures may be birefringent under the proper conditions. The effect is frequently due to parallel orientation of protein fibers with their long axes perpendicular to the light beam. This orientation appears to persist in structures such as muscle fibers, chromosomes (in condensed stages), myofibrils, and cilia. In undifferentiated living cytoplasm birefringence is common but highly variable in degree, and may be elicited by dehydration, mechanical stress, and chemical treatment. Here, too, protein fibers seem to be frequently responsible for the optical property.

On purely theoretical grounds, these results suggest that the nucleic acids in cell structures may exist as aggregates so oriented as to elicit the absorption anomalies. Astbury and Bell (*2*) have shown that the internucleotide spacing of the nucleic acid molecule is practically identical with the spacing of side-chains along the protein polypeptide skeleton. This suggests that the nucleoprotein complex probably involves a side-by-side binding of the thread-like protein molecules with the equally elongated nucleic acids. This would mean that in any structure containing nucleic acids these would be oriented in the same way and to the same degree as the proteins with which they are combined. This occurs in tobacco mosaic virus (*3*).

Direct evidence on this possibility is difficult to obtain from measurements of birefringence. In a few cases, such as sperm heads, the nucleic acid content is sufficiently great to give the entire structure negative birefringence, indicating that the nucleic acid is in an oriented state. In most structures, however, the nucleic acids are present in small concentrations in comparison with protein. The negative birefringence caused by the nucleic acids thus cannot counteract the large opposite effect due to the protein present. As Caspersson (*7*) has pointed out, the birefringence expected from the usual concentrations of nucleic acid lies close to the limits of the method. Hence, the failure to detect negative birefringence, where the proteins are themselves oriented, cannot be taken as evidence that the nucleic acids are not oriented.

It is not surprising, then, that the only unequivocal evidence for orientation of nucleic acids comes from studies of sperm heads. Schmidt (*27*) detected a strong negative birefringence in this case, and concluded that the nucleic acid molecules were oriented

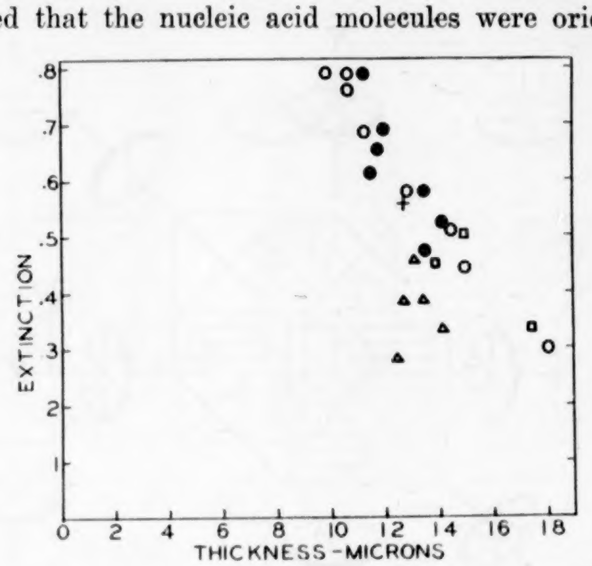

FIG. 2. The relation between extinction at 257 mμ and the thickness of nuclei of spermatocytes of *Gomphocerus*. Data taken from Table 1 of reference *5*. Triangles represent nuclei in early leptotene; open circles—late leptotene; plus sign—synizesis; closed circles—pachytene; squares—diplotene.

in lines parallel to the long axes of the protein fibers. This was confirmed by Caspersson's observation of dichroism at 257 mμ in the sperm bundles of a locust (*7*).

The evidence for the orientation of nucleic acid in chromosomes is contradictory. Schmidt (*27*) found that the chromomere discs of salivary gland chromosomes have a weak negative birefringence, and concluded that the nucleic acid was oriented as in sperm heads. However, Frey-Wyssling (*14*) concludes from quantitative determinations of the optical properties of the chromomeres that only a small percent of the nucleic acid molecules are oriented. Furthermore, Caspersson (*7*) failed to find the dichroism at 257 mμ expected from Schmidt's conclusions. Caspersson suggests that the insect salivary gland chromosome is not of typical composition, and he does not consider these data sufficient to rule out the probability that nucleic acids are oriented in most chromosomes. Negative birefringence has been demonstrated in chromosomes of a number of animal and plant cells, as well as in "resting" nuclei. This property is variable in degree, and marked changes (including changes in sign) may result from environmental effects and fixation procedures.

Extinctions yielded by cellular nucleic acids.

Since both conditions required for the appearance of nonlinear relations between E and N (i.e., absorption due to a distinctive axis, and orientation)

may apply to the intracellular nucleic acids, it is of interest to examine the data obtained from such objects for evidence of this anomaly.

Demonstrations that intracellular extinctions at 257–260 mμ do indeed follow the Beer-Lambert law

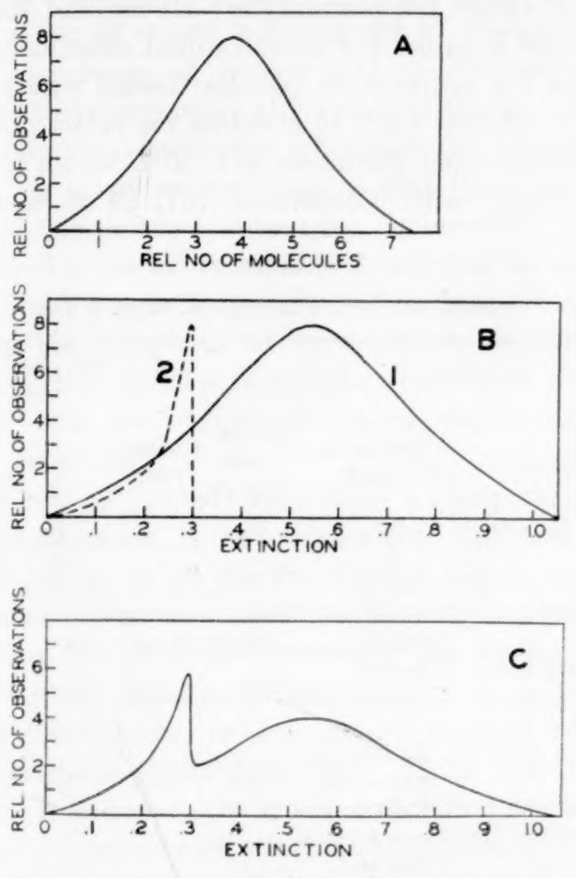

FIG. 3. The effect of orientation on the distribution frequency of extinction values. Fig. 3A is an arbitrarily drawn normal distribution curve for the actual number of nucleic acid molecules in a significantly large population of similar cellular structures. Fig. 3B shows the distribution curves for the extinction values calculated from the relative number of molecules according to the E vs. N equation for unoriented material (solid line) and oriented material (broken line). Fig. 3C is the distribution curve expected from a population containing equal numbers of oriented and unoriented objects.

would dismiss the possibility that the orientation effect is of any consequence. As far as the writer is aware, there are only two published descriptions of a direct test of this relationship in cells, and neither involves naturally-occurring solid structures. Pollister and Ris (25) have shown that the extinction yielded by the colored substance produced in the Millon test for proteins is directly proportional to the thickness of the test section. Since the structure of the compound is unknown, it is impossible to say whether it is sufficiently nonsymmetrical to produce the orientation effect. There is no evidence that the colored substance becomes oriented by whatever structural organization exists in the proteins tested. Commoner (*11*) found that the extinction due to anthocyanins and flavones dissolved in intracellular fluid vacuoles is proportional to the vacuole thickness. In this case, the orientation effect is not expected, since the absorbing substance is in solution and therefore completely unoriented. It should be noted that both of these studies test only Lambert's law (linear relation between E and sample thickness). The relation of E to the number of molecules per unit sample thickness was not determined.

One of Caspersson's papers (*5*) does include a table relating E at 257 mμ to the thickness of nuclei of *Gomphocerus* spermatocytes in various stages of prophase. These data have been plotted in Fig. 2, and strikingly fail to follow the Beer-Lambert laws. Indeed, the extinction appears to be inversely proportional to the sample thickness.

In the absence of experimental demonstration that the extinction resulting from the nucleic acids of cell structures is actually proportional to the number of molecules scanned, the validity of interpreting such measurements according to the Beer-Lambert law remains an open question. From certain of the extinction data in the literature, it is possible, however, to obtain an indirect test of the linearity of the E to N relationship. Although this method cannot be applied to all the available extinction data, the results obtained from those cases susceptible to this type of analysis suggest that the orientation effect may play an important rôle in determining intracellular extinctions due to nucleic acids.

This analysis is derived from a consideration of the curve relating E to N for the case of fully oriented nucleic acid molecules. (See Fig. 1 of paper *12*, page 43.) This curve shows that *all* oriented objects containing more than a relatively small number of nucleic acid molecules yield the value $E = 0.3$. Since all such objects give the same E value, regardless of nucleic acid content, it is to be expected that the frequency distribution of E values for a significantly large group of structures containing nucleic acid will be distorted by this phenomenon.

This is illustrated in Fig. 3. Assume that in any large population of cytological structures, the *actual* nucleic acid content (N) of each object in the group follows some normal Gaussian distribution curve. An example of such a curve, arbitrarily drawn, is shown in Fig. 3A. Now if this population of objects is examined spectrophotometrically, the E values obtained will yield a frequency curve *the shape of which depends on whether or not the objects contain oriented material.* If all objects in the population are unoriented, E is proportional to N for all values of N. Curve 1 of Fig. 3B is the frequency distribution of the E values calculated from the population shown in Fig. 3A, according to this proportionality.

On the other hand, if the whole population is com-

posed of fully oriented structures, E is proportional not to N but to the expression $0.3\text{-}\log_{10}(e^{-aN}+1)$.[4] If E values are calculated from the N values of curve 3A according to this relationship, and plotted against the corresponding frequency, the distribution curve 2 of Fig. 3B is obtained. It is impossible for such a population to yield any E values above 0.3. If the population contains equal proportions of unoriented and fully oriented structures, the distribution curve shown in Fig. 3C will result (obtained by adding curves 1 and 2).

Fig. 3C shows that if a significant fraction of a population of cytological elements contains fully oriented nucleic acid molecules, a rather unusual type of frequency curve will be found for the E values obtained. There will be a tendency for the values between $E=0.2$ and $E=0.3$ to predominate, and the frequency of observations will drop sharply as E rises above 0.3. At higher E values a secondary maximum may appear. If the proportion of oriented objects is very low, this second maximum may be the dominant one, but in any case the curve shows an unusual number of values between 0.2 and 0.3, with a sharp drop just above this range.

This general relationship will not be altered by differences in the direction from which a cytological structure is viewed, for as shown above, the value of $E=0.3$ is limiting, regardless of the direction of the incident beam. If the assumption made previously, that the absorption at 260 mμ is due to a distinctive axis, is not true, then the relative frequency with which values around 0.3 would occur would be reduced. However, values in the range $E=0.2\text{–}0.3$ would still occur with an unusual frequency, and the qualitative effect on the distribution frequency will not be altered.

This type of analysis can be applied to extinctions obtained from cell structures if the following conditions are met: (1) The data must comprise a significantly large series of extinction measurements made on similar structures, and (2) the size of the field scanned must be so small as to cover a homogeneous area within the structure (if the field is large, differences in the spatial organization of various areas included in it may obscure the orientation effect). If such data yield frequency distributions (i.e., of E values) which are distorted by a predominance of values in the range $E=0.2\text{–}0.3$, it is suggested that the group of structures examined contain a significant number of objects with oriented nucleic acid. The E values obtained in these sets of data are therefore not necessarily proportional to nucleic acid content.

The method is applicable to the following published studies on extinctions due to cellular nucleic acids, all of which are based on scanning areas of the order of 1 square micron and include at least 29 measurements on similar material: Thorell (*32*), 132 E values for erythroblasts; Thorell, Bing, and Fagraeus (*33*), 117 E values for plasmacytes; Hydèn and Hartelius (*18*), 30 E values for normal rabbit neurones, and 28 values for neurones of animals treated with malononitrile; Hydèn (*17*), 46 E values for rabbit neurones; Caspersson and Santesson (*9*), 30 E values for various tumor cells; Caspersson (*6*), 29 E values for

TABLE 1

SOURCE OF DATA PLOTTED IN FIGS. 4 AND 5

Reference	Type of cell	Thickness in μ	Condition	No. of extinction values at 257–260 mμ: cytoplasm	nucleus	total
Thorell (*32*)	Rabbit erythroblasts	10–16	living & fixed	143	8	151
Thorell, Bing, and Fragaeus (*33*)	Rabbit plasmacytes	10–16	living	117	0	117
Hyden (*17*)	Rabbit neurones	5	fixed	28	25	53
Hydèn and Hartelius (*18*)	Rabbit neurones—normal and malononitrile-treated	5–10	fixed	58	0	58
Caspersson (*6*)	Insect salivary gland cells	2–3	fixed	0	29*	29
Caspersson and Santesson (*9*)	Tumor cells	5	fixed	22	8	30

* Includes 16 readings on chromosome structures, 3 on nuclear sap, 10 on nucleoli.

various regions of nuclei and chromosomes.[5] Details concerning the conditions of these measurements are given in Table 1.

The frequency distributions obtained from the data presented in each of these studies are shown in Figs. 4 and 5. The extinction values were taken from tables published in these studies, or read off the published absorption spectra. The tabular values are for extinctions at 257 mμ; the other values are for 260 mμ, at which the absorption spectra usually indicate a measured point.

Of the seven sets of measurements, three (Figs. 4B, 4C, and 5, solid curve) yield distribution curves which show definite maxima at $E=0.2\text{–}0.3$. One curve (Fig. 4D) which shows a maximum at this range is not si

[4] a = absorption coefficient per molecule.

[5] Consideration of a significantly large set of measurements of neurones by Gersh and Bodian (*15*) has been excluded because the scanning area used was so large as to include a heterogeneous section of the cell.

nificant in itself because of the smallness of the population, but is presented for the sake of completeness. Two sets of data (Figs. 4A and 5, broken curve) show maxima at E values significantly greater than 0.3. The set of data on nuclear structures (Fig. 4E), while too small to be statistically significant, shows a tendency for E values less than 0.3 to predominate.

The occurrence of several sets of measurements with well-defined maxima at 0.3 suggest that the orientation effect has probably influenced the values obtained. It is, of course, possible that these maxima reflect actual nucleic acid contents (unoriented) which correspond to the extinction of 0.3, and that the occurrence of this particular number of nucleic acid molecules in the cytoplasm of erythroblasts varying in thickness from 10–16 μ, in sections of neurones 5–10 μ thick, and in 5-μ sections of various tumor cells is purely fortuitous.

Detailed analysis of these data suggest that the latter explanation is not admissible, and that the maxima shown in *all* the distribution curves can be more reasonably accounted for by the orientation effect than by actual differences in the nucleic acid content of the objects studied.

In the first place, it is necessary to consider the thickness of the materials studied. If the Beer-Lambert law is followed, the modal extinctions would be expected to increase with increasing thickness, unless differences in nucleic acid content just happened to cancel out this effect. Actually, as can be seen from Table 1, the value $E = 0.3$ tends to be limiting despite rather large variations in thickness of the materials studied. The four sets of data yielding maxima at 0.3 were obtained from material 5 μ, 5 μ, 5–10 μ, and 10–16 μ respectively. The measurements made on the thinnest sections (nuclear structures of 2 μ thickness) give the only modal E value which is less than 0.3. These observations conform with the relationships expected from the orientation effect. In oriented material E can vary with N only when the number of molecules contained in the object is so low as to give extinctions less than about 0.3; with increasing values of N (e.g., due to increasing thickness of samples) E remains at the limit of 0.3 imposed by the orientation phenomenon.

Consideration of the measurements made on plasma cells, which show a modal E value of 0.5, provides further evidence on the orientation effect, and suggests that the optical properties of the nucleic acids in undifferentiated cytoplasm are largely dependent on whether or not the cell studied is living or dead.

The plasmacytes (Fig. 4A) represent the only group of material comprised of cells which were apparently alive at the time of measurement. The cells

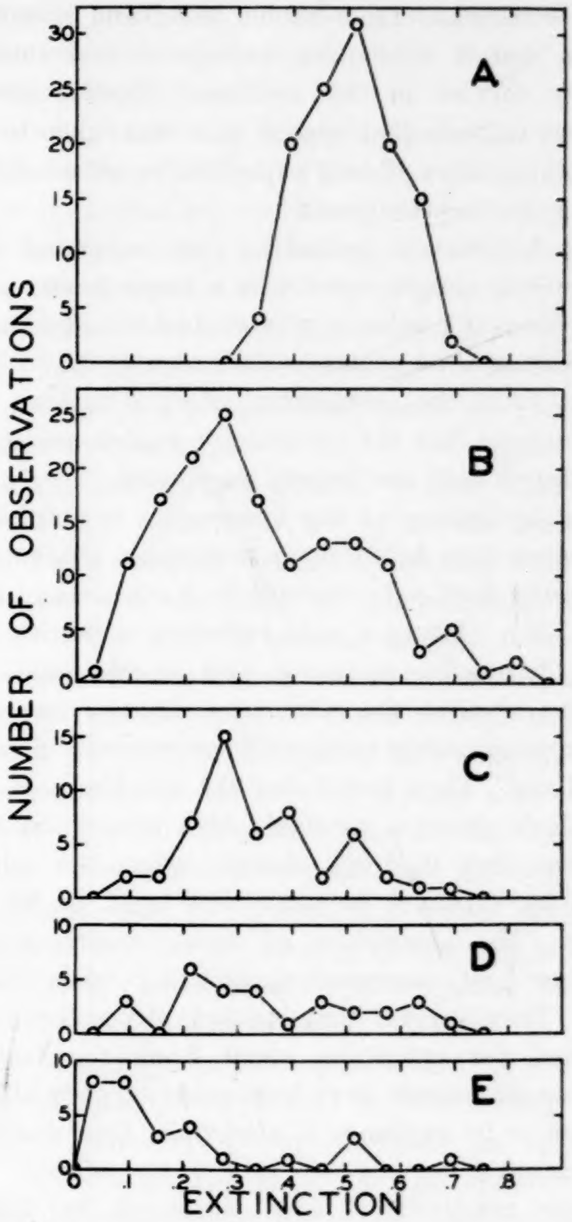

Fig. 4. Distribution frequencies for the extinctions at 257–260 mμ given by various cytological structures. See Table 1 for details of the conditions for measurements. A—Living rabbit plasmacytes (data taken from reference *33*); B—an apparently mixed population of living and dead rabbit erythroblasts (data taken from reference *32*); C—rabbit neurones, fixed (data taken from reference *17*); D—various tumor cells, fixed (data taken from reference *9*); E—various nuclear structures, fixed (data taken from reference *6*).

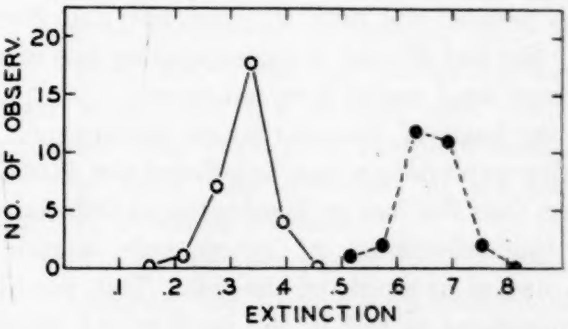

Fig. 5. Distribution frequencies for the extinctions at 257–260 mμ given by neurones from normal rabbits (solid line) and rabbits treated with malononitrile (broken line). (Data taken from reference *18*.) See Table 1 for further details.

used were taken from rabbit blood and mounted in saline, and it is common experience that such cells readily survive in this medium. Furthermore, the authors indicate that special care was taken to avoid the killing effect of long exposures to ultraviolet light during the measurements.

The distribution yielded by these surviving cells is a relatively simple curve with a single peak at about 0.5. Since this value is greater than 0.3, and since the predominance of values in the range of 0.2 to 0.3 expected from the orientation effect is lacking, these data suggest that *the cytoplasmic nucleic acids of living plasma cells are largely unoriented.*

The significance of this observation is amplified by the comparison between the ultraviolet absorption of living and dead cells reported by Larionov and Brumberg (*20*). Using a new reflecting objective which made it possible to focus with visible light, these workers avoided the ultraviolet damage incident to most measurements made with conventional ultraviolet objectives. They found that the cytoplasm of living fibroblasts shows a relatively high absorption at 260 mμ, but that it drops sharply when the cells are killed by exposure to ultraviolet light, or by other means. The absorption of living nuclei, however, was low and increased significantly when the cell died. They suggest that the low absorption usually reported for cytoplasm stems from the fact that most measurements have been made on cells killed by fixation or by exposure to ultraviolet light during the experiment.

These results have been confirmed by Ris and Mirsky (*26*), who found that photographs of living chick fibroblasts taken at 2537 A showed strong absorption in the cytoplasm, which decreased markedly when the same cells were killed by exposure to ultraviolet light. These authors suggest that the loss of ultraviolet absorption on death is a result of "ribosenucleic acid leaking out of the disintegrating cell." This explanation seems doubtful, however, for the very large nucleic acid molecules would diffuse slowly, and as pointed out both by Larionov and Brumberg and by Ris and Mirsky, a corresponding loss of nucleic acid from dead nuclei does not occur.

On the basis of the orientation phenomenon an alternative explanation can be offered for this effect—namely, that the loss in absorption at 260 mμ results from the orientation of cytoplasmic nucleic acids which occurs at death of the cell. This would cause the extinctions to fall to the limit of 0.3 despite the fact that the cytoplasm might contain sufficient nucleic acid to give higher extinctions in the unoriented state. This view is supported by observations (*27*) that sharp increases in the birefringence of cytoplasm may be induced by fixing agents.

The bimodality of the distribution curve for erythroblasts (Fig. 4B) may be explained similarly. The predominance of values in the range 0.2 to 0.3 suggests that a large fraction of the measured cells contained oriented cytoplasmic nucleic acids. But the appearance of the secondary maximum at 0.5 would indicate that at least some of the cells contained unoriented cytoplasmic nucleic acids. It would be expected, therefore, that the population of erythroblasts consisted of a mixture of living and dead cells at the time of measurement (cf. Fig. 3C).

The nature and treatment of the erythroblasts is in accord with this expectation. Thorell reports that the erythroblasts were obtained from living bone marrow, teased out in saline, and mounted on slides under cover glasses sealed with vaseline to prevent drying. Although care was taken to avoid killing by undue exposure to ultraviolet light, the survival time of teased-out bone marrow cells in a thin sealed film is probably limited; and one would anticipate that some fraction of the cells were dead when measured. Furthermore, some of Thorell's preparations were treated with lanthanum acetate to precipitate the nucleic acids and provide a "more stable preparation" (page 46, reference *32*). Since such cells were dead, this treatment apparently contributed to the size of the nonliving fraction of the population. The bimodal character of the frequency distribution given by the erythroblasts may therefore reflect the fact that some of the cells measured were living (unoriented cytoplasmic nucleic acids, modal $E = 0.5$) and others dead (oriented cytoplasmic nucleic acids, modal $E = 0.3$).

The data are thus consistent with the idea that the nucleic acids in the undifferentiated cytoplasm of living erythroblasts and plasmocytes are unoriented, therefore yielding extinction values which are relatively high and probably proportional to the actual nucleic acid content. On the other hand, the cytoplasmic nucleic acids of dead cells tend to become oriented, thus yielding extinction values which fail to exceed 0.3 and so are independent of nucleic acid contents which would, if unoriented, give higher E values.

It is possible that the changes in the ultraviolet absorption of nuclei on death which have been noted (*20, 24, 26*) may be due to similar variations in the degree of orientation imposed on the nucleic acids. However, the available data are too incomplete to provide an adequate basis for an analysis similar to the one outlined, and the question must remain open at present.

The increase in extinction at 260 mμ of neurones from rabbits treated with malononitrile (Fig. 5) is interpreted by Hydèn and Hartelius (*18*) as representing a proportional rise in the nucleic acid content

of these cells. However, the ultraviolet photographs of the treated cells show that malononitrile causes a marked change in the configuration of the structures, which are apparently rich in nucleic acid (the Nissl bodies). In the untreated cells, the Nissl substance has the "tigroid" appearance of more or less clumped material. Cells from malononitrile-treated animals, on the other hand, show a structureless dense mass diffusely spread through the cytoplasm. It seems likely, therefore, that the treatment induces a significant change in the degree of aggregation of the Nissl substance. Since the aggregated (normal) form of Nissl substance gives modal E values of about 0.3, there is reason to believe that it may contain oriented nucleic acids. It seems possible that the increase in the modal extinctions exhibited by the treated material may be a result of disaggregation (and the concomitant disorientation) of nucleic acids caused by malononitrile. The rise in extinction induced by the treatment would not reflect an actual increase in nucleic acid content.

This analysis suggests that differences between the extinction at 260 mμ of cellular structures may frequently arise from variations in the degree of orientation of their constituent nucleic acids, rather than from real differences in nucleic acid content. Thus, where structures differ considerably in the degree of orientation of their nucleic acids, failure to consider this factor may lead to large errors in interpretation.

For example, a study of the extinction of nuclei in various stages of spermatogenesis in the locust has led to the conclusion (*4*) that a "considerable amount of nucleic acid disappears from the nucleus" as the sperm ripens. The reduction in extinction is evident from ultraviolet photographs (reference *4*, Figs. 79, 80) in which spermatocytes show considerably darker nuclei than do neighboring sperm cells. However, the two cell types also differ greatly in the degree of orientation of their nuclear material. During sperm formation, the spherical nucleus of the spermatocyte stretches into the elongated spindle form which characterizes the mature sperm. Optical studies (*23, 27*) show that the birefringence of spermatid nuclei increases in parallel with elongation, and that the nucleic acid molecules thereby become markedly oriented. This orientation has been directly demonstrated (*7*) in photographs of sperm bundles taken with ultraviolet light (260 mμ) of varying planes of polarization. The material is highly dichroic, yielding a maximum extinction when the plane of polarization is perpendicular to the long axis of the sperm nuclei. It seems likely, therefore, that the reduction in ultraviolet extinction accompanying the elongation and maturation of the sperm nuclei is not due to "loss" of nucleic acid, but is rather a consequence of the orientation which is imposed on the nuclear material during spermatogenesis.

A similar case is the determination of the extinction of muscle fibers (*10*). The E values for isotropic segments of striated muscle fibers are significantly higher than those given by neighboring anisotropic segments. Since the absorption at 257 mμ is probably related to the adenylic acid (and its phosphorylated compounds) of the muscle, this observation led to the conclusion that most of the adenine compounds are localized in the isotropic segments.

An alternative interpretation of these data may be offered. The anisotropy of the muscle fibers seems to be due to oriented micelles of myosin. The myosin fibers are also the locus of the adenosine triphosphatase activity of the muscle, and the formation of a complex between myosin and adenosine triphosphate has been demonstrated. It seems possible that a significant part of the nucleotide in muscle fibers will be oriented by attachment to myosin wherever this protein is itself oriented (i.e., in the anisotropic bands). Hence the isotropic bands' showing greater extinctions than the anisotropic bands may result from the fact that the adenine compounds tend to be more strongly oriented in the latter, rather than from any difference in concentration.

The foregoing analysis suggests that the orientation effect probably is important in determining the extinctions given by cytological structures at 257–260 mμ. The ultraviolet absorption of such structures cannot be evaluated by the simple application of the Beer-Lambert laws. The interpretation of intracellular extinction values at 257–260 mμ seems to be subject to the following reservations:

1) Where part or all of the nucleic acid in a structure is oriented in three dimensions, E is not proportional to the nucleic acid content unless E is smaller than about 0.1–0.2.

2) In general, the E values yielded by oriented aggregates may be considerably smaller than the E value which the same number of nucleic acid molecules would yield if not oriented; but this relationship is reversed when very low nucleic acid contents are encountered. For the case of complete orientation, E cannot rise above 0.3, regardless of the number of nucleic acid molecules scanned.

3) If the orientation effect occurs, it is not possible to calculate the nucleic acid content of a cell structure by comparing its extinction with the extinction given by a known number of nucleic acid molecules in solution.

4) In comparing two cytological structures, it would appear impossible to obtain a ratio of their nu-

cleic acid contents from the ratio of their extinction values (for equal thicknesses) unless it can be shown that the same degree of orientation occurs in both structures.

These reservations apply to the interpretation of qualitative observations, such as the darkness of ultraviolet photographs, as well as to the treatment of quantitative photoelectrical measurements.

The analysis presented is suggestive rather than conclusive. It does, however, indicate that the entire subject needs to be more closely scrutinized before it is possible to accept the assumptions on which many studies of ultraviolet absorption are based, or to look upon the resulting conclusions (*8*) as a sound foundation for theories of protein metabolism, gene action, and the special roles of the nucleic acids.

The need for new data is apparent. The location of the absorption axes of the nitrogen bases must be known if the optical behavior of nucleic acids is to be fully understood. Determination of dichroism at 260 mμ could yield precise information on the orientation of nucleic acids. The validity of Lambert's law could be determined by studying the extinctions of various thicknesses of the same or similar structures. Some way needs to be found for testing Beer's law within cells. The optical changes which occur on the death of the cell seem to be sufficiently important to warrant much attention.

Structural considerations other than orientation proper may be related to the light-absorbing powers of cellular substances. The work of Sheppard, Scheibe (see reference *12* for references), and others shows that polymerization and simple aggregation may cause serious changes in the absorption characteristics of certain substances. Such effects may also induce anomalous behavior in nucleic acid solutions and may account for the recent observation (*19*) that polymerization of nucleic acid causes a significant change in extinction coefficient.

The optical consequences of orientation of cellular materials may play an important role in determining the properties of photochemical systems in living cells. There is evidence (*29*) that the degree of aggregation of some substances may determine the appearance of new absorption maxima which result from intermolecular electron transfers. The fundamental importance of such molecular behavior in living systems has been suggested by the work of Szent-Györgyi (*31*).

The value of the application of optical methods to cellular biology is apparent from the important results already achieved. It may not be amiss to suggest, however, that the full use of this tool depends largely on an understanding that we are dealing with a complicated and dynamic state of matter, in which relationships inconsequential in simpler systems become of decisive importance. Close attention to the special and uncommon optical properties of living cells may be more revealing than their similarities to the states of matter which are dead.

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TECHNICAL PAPERS

Chromosome Numbers for Two Species of Mexican Commelinaceae

Earlene Atchison, H. E. Moore, Jr., and C. E. Wood, Jr.

University of North Carolina,
Bailey Hortorium at Cornell University, and
University of California

Although the Commelinaceae have provided classic material for cytological and cytotaxonomic studies (*1–3*), sufficient material has never been available for thorough treatment of the Central American representatives of the family. The authors report the following collections from Mexico:

Tradescantia iridescens Booth ex Lindl. $n = 6$. Borders of oak woods near Real del Monte, State of Hidalgo, Mexico. Altitude, 8500–8700 ft. Aug. 1, 1948. *H. E. Moore, Jr., C. E. Wood, Jr., E. Atchison, 4247.*

FIG. 1. (a) *T. iridescens*, pollen mitosis, $n = 6$. (b) *W. candida*, root-tip mitosis, $2n = 24$.

Weldenia candida Schult. f. $2n = 24$. Wet pockets on sides of granitic rock masses above Pueblo Nuevo, road from Real del Monte to El Chico, State of Hidalgo, Mexico. Altitude, 9000–9500 ft. July 25, 1948. *H. E. Moore, Jr.,* and *C. E. Wood, Jr., 4082.*

T. iridescens, $n = 6$ (Fig. 1a), is of particular cytotaxonomic interest since it is the first diploid species to be reported among the Mexican tradescantias. The species is apparently endemic to the area around Real del Monte; it is known only from the type collection, *H. E. Moore, Jr., 3111*, and the collection cited above. *W. candida*, $2n = 24$ (Fig. 1b) is found in the alpine regions of central Mexico and Guatemala.

Specimens will be deposited in the Bailey Hortorium, Gray Herbarium, and Museum of the Department of Agriculture, San Jacinto, D.F., Mexico.

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The Application of the Beer-Lambert Law to Optically Anisotropic Systems

Barry Commoner and David Lipkin

Henry Shaw School of Botany and
Department of Chemistry, Washington University

The determination of the concentrations of various substances in cell structures by the measurement of their absorption spectra in the cell is becoming a widely used experimental procedure (*3, 4, 6, 11*). Caspersson and his co-workers (*3*), for example, have made quantitative estimates of the relative, and sometimes absolute, concentrations of nucleic acids, proteins, and other substances in cells by the use of this technique.

The quantitative evalution of such microspectrophotometric measurements is dependent on the validity of the assumption that the decrease in intensity of a beam of monochromatic light on passing through a cell structure is quantitatively related to the number of molecules of absorbing material in the path of the light beam. Recognizing this limitation, Caspersson has shown (*1*) that in the passage of a light beam through microheterogeneous structures such as cytoplasm, the losses due to nonabsorption processes are relatively small and calculable. This consideration of nonspecific light losses, however, does not accomplish the complete validation of intracellular extinction measurements as an index of concentration. Even though the difference in intensities of the incident and transmitted light beams may actually be due to absorption of photons by a given cell constituent, it is still necessary to know the quantitative relationship between the amount of light absorbed and the number of molecules in the path of the light beam. Usually it is assumed that this relationship is described by the Beer-Lambert law, $E = \log I_0/I = \varepsilon cd$.[1]

This law may be derived using the following assumptions: (a) that a single molecular species is absorbing the light, and (b) that variations in concentration do not affect the interactions either between solute molecules or between solute and solvent in such a way that the prob-

[1] E = extinction, I_0 = intensity of the incident light beam. I = intensity of the transmitted beam, c = concentration of absorbing material in moles/liter, d = thickness of the sample in cm, and ε = molar extinction coefficient.

ability of absorption of a light quantum by a single solute molecule is changed. No published data are available on the applicability of the Beer-Lambert law to light absorption by molecules in the complicated states of aggregation characteristic of cell structures. Since interpretations of absorption measurements (e.g., by Caspersson and co-workers) are based on the above proportionality between c and E, and because the theoretical conclusions derived from them have been of considerable interest to biologists, it is necessary to consider to what degree the Beer-Lambert law is valid for the actual conditions of intracellular measurements. It is the purpose of this paper to examine one of these conditions—the orientation of the absorbing molecules—for its effect on the validity of the Beer-Lambert law.

It has been suggested (*9*) that the absorption by a molecule of a light quantum of appropriate energy is due to the excitation of electronic oscillations in the molecule. Except in the case of an optically symmetrical molecule, the probability of absorption of a given quantum depends on the spatial relationship between the molecule and the electric vector of the incident light. Specifically, this probability is proportional to $\cos^2 \theta$, where θ is the angle between the direction of the electronic oscillation (an optical axis of the molecule) and the electric vector of the light beam. Consequently, the probability of absorption is zero when the electric vector is perpendicular to the optical axis and increases to a maximum as the angle becomes zero. The effect of orientation on the absorption spectra of optically anisotropic molecules has been studied by Weigert (*16*), Taylor (*15*), Scheibe (*12*), Lewis and co-workers (*10*), Jelley (7), and Sheppard (*14*). If such molecules are examined with unpolarized light in dilute solution they show no unusual optical properties. In this case the electric vectors of the light beam are randomly distributed in a plane at right angles to the beam and the dissolved molecules are oriented at random. On the other hand, if the optically anisotropic molecules are oriented and examined with plane polarized light, then they exhibit dichroism: the extinction varies with the orientation of the plane of polarization. Caspersson (*2*) has shown that partially oriented films of thymonucleic acid exhibit this behavior at 265 mμ. Furthermore, the work of Schmidt (*13*), Frey-Wyssling (*5*), and others indicates that the nucleic acids in cell structures may be oriented to a greater or lesser degree. The same is true of other optically anisotropic substances occurring in cell structures.

Since practically all cellular extinction values are obtained with unpolarized light, one might expect that the above factor would not interfere with the measurements. As we shall show below, such an expectation is unwarranted. A consideration of the quantitative relationship between the number of molecules in the light beam and the extinction (E) shows that this function is dependent on the degree of orientation of the optical axes of the molecules *even when the measurements are made with unpolarized light.*

We shall limit our considerations to a molecule in which there is possible a transition which corresponds to a distinctive axis. In other words, the molecule must have sufficiently low symmetry so that it has one optical axis which is different from all of the other possible optical axes. The cyanine dyes fall into this category; the optical axis corresponding to the main absorption band of longest wavelength coincides with the largest dimension of the molecule (*9*). Malachite green also falls into this group. It has three different optical axes; one corresponds to the absorption band at 625 mμ and another to the band at 423 mμ (*8*). It should be noted that crystal violet also has three optical axes, but that two of them are identical. The discussion which follows would pertain only to the one axis which is distinctive. The purines and pyrimidines would, like malachite green, have three different optical axes. The following comments apply here to absorption corresponding to any one axis.

The relationships between the extinction (E) for *unpolarized light* and the number of molecules of the type described above have been derived for three degrees of orientation of a given optical axis relative to the light beam, as follows:[2]

Case I. Completely random orientation of the optical axes. In this case the relationship is

$$\log_e \frac{I_0}{I} = \frac{\alpha N}{3}, \text{ or } E = \frac{\alpha N}{3 \times 2.303}.^{3}$$

Case II. The optical axes all lie in a stack of planes with each plane perpendicular to the light beam, but the axes in each plane are randomly oriented. Here the relationship takes the form

$$\log_e \frac{I_0}{I} = \frac{\alpha N}{2}, \text{ or } E = \frac{\alpha N}{2 \times 2.303}.$$

Case III. The optical axes are all parallel and the light beam is in a plane perpendicular to these axes. The relationship now becomes $\log_e \dfrac{I_0}{2I - I_0} = \alpha N$, or $E = 0.3 - \log_e (e^{-\alpha N} + 1)$.

These three functions are plotted in Fig. 1. It can be seen that for the case of a completely oriented group of molecules (Curve 3, Case III) E is approximately proportional to the number of molecules only at extinction values less than 0.15. Above this value the slope of the curve changes until it approaches zero, so that as E approaches 0.3 the amount of light absorbed becomes independent of the number of molecules. In other words, if a completely oriented aggregate of optically anisotropic molecules such as we have described above is examined with monochromatic unpolarized light, then the amount of incident light absorbed can never be above 50%. The same type of behavior is exhibited by a sheet of Polaroid. If we consider a group of optically anisotropic molecules whose orientation corresponds to Case II (see Curve 2, Fig. 1), the relationship between E and N is a linear one but not quantitatively the same as if the molecules were randomly oriented. For a given number of molecules E for random orientation in a plane is 50% greater than

[2] We are indebted to Prof. S. I. Weissman for the derivation of these equations and for many helpful discussions of the problem.

[3] In these equations N = the number of molecules per unit area of light beam, and α = the absorption coefficient per molecule when the optical axis of the molecule and the electric vector of the light beam are parallel.

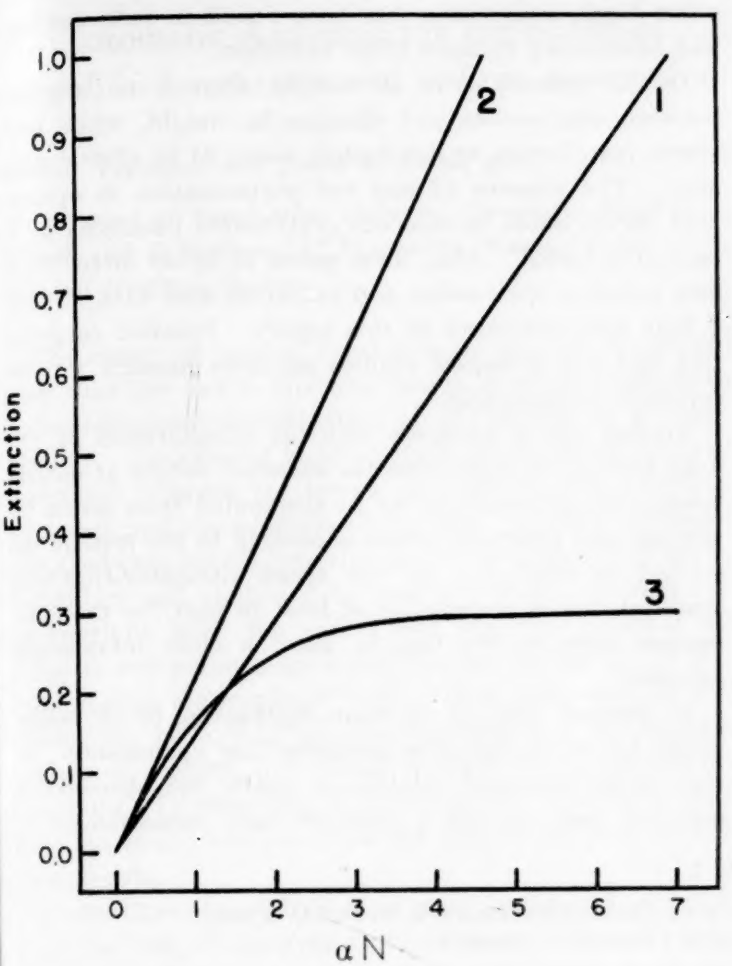

FIG. 1.

E for random orientation in three dimensions. If similar calculations are made for samples in which only a part of the absorbing molecules are fully oriented, one obtains curves lying between Curves 1 and 3 (Fig. 1). These considerations lead us to the conclusion that the Beer-Lambert law must be applied with caution to groups of optically anisotropic molecules which may be more or less oriented.

As previously noted, there is evidence for some degree of orientation in practically all cytological structures other than liquid vacuoles (*5, 13*). Further, most biologically important molecules are optically anisotropic. Hence the assumption that the concentration of such an absorbing substance in a cell structure can be calculated from the ratio of its extinction value to the extinction of a given number of molecules of the substance in solution is not valid. Furthermore, unless the orientation of the optical axes in two cytological structures is identical, the assumption that the ratio of their extinctions per unit thickness is equal to the ratio of their content of such absorbing material is also invalid. Thus, in cytological absorption measurements made with unpolarized light, variations in extinction values may arise from differences in the degree of orientation rather than from differences in content of any specific substance. It seems clear that the entire problem of interpreting intracellular extinction measurements needs to be reexamined with the realization that one is dealing not with true solutions but with oriented aggregates of molecules.

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Opalescence of Serum after Total Body X-Irradiation as a Prognostic Sign of Death[1]

Robert L. Rosenthal[2]

Radiation Laboratory and Division of Medical Physics, University of California, Berkeley

During an investigation of the clotting reaction of blood after irradiation with 200-kv X-rays, the author noted the appearance of a marked opalescence in the serum and plasma of rabbits, which subsequently died a few days after exposure to a single lethal dose of total body irradiation. This opalescence appeared within 24 hr following the exposure to radiation. In all cases, it disappeared completely 72 hr after exposure. A review of the literature has failed to reveal any mention of this phenomenon.

Rabbits of the New Zealand white strain were given over the total body single doses of 200 kv X-irradiation, calibrated in air by a Victoreen ionization chamber. Dosage ranged from 200 to 1000 roentgens (r). Blood samples were obtained by cardiac puncture before radiation and at various intervals after radiation (up to 30 days). Serum was obtained from the clotted blood and plasma was obtained by centrifugation of either citrated or oxalated blood. In all cases the opalescence, when present, was noted in both serum and plasma.

The opalescence was noticeable as a pearly white tint homogeneously distributed throughout the sample. Various degrees of intensity have occurred and can be classified as marked, moderate, and slight, as shown in Fig. 1. All animals showing marked opalescence died as a

[1] Portions of this study were aided by the Atomic Energy Commission and U. S. Navy Contract N6Oori-111, Task Order III Project No. NR 171-138.

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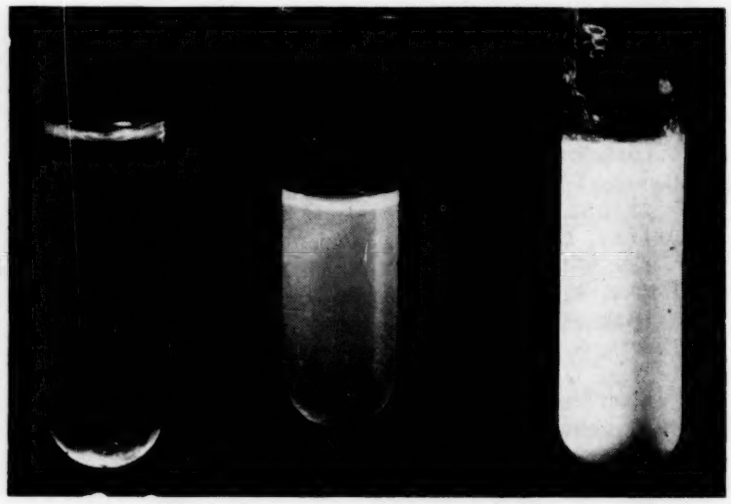

FIG. 1. Tube at left shows clear serum as found before irradiation of rabbit. Center tube shows moderately opalescent serum (rabbit No. 16, 24 hr after irradiation; animal sacrificed 34 days after irradiation). Tube at right shows markedly opalescent serum (rabbit No. 15, 24 hr after irradiation; died a few hours later).

result of radiation within 5 days following exposure. Animals having no opalescence or opalescence to a lesser degree usually survived the radiation for at least 30 days, unless death occurred from other causes.

If opalescence occurred it was prominent 24 hr after radiation, and completely disappeared 3 days after radiation in all cases. No relation was noted between its occurrence and diet or fasting. Serum obtained either with or without fasting (20 hr) was always clear except for the opalescence following radiation. On the other hand, opalescence has been found in both fasting and nonfasting animals after radiation.

In the present series of rabbits, there is no relation between opalescence and changes in weight, white cell count, lymphocyte, or heterophile count 24 hr after radiation. The absence of any red pigmentation in opalescent serum tends to rule out erythrocyte hemolysis as a causative factor. Also, there seems to be no direct relation between opalescence and radiation dose rate. Table 1 lists data pertinent to this report. Detailed coagulation and hematological studies on these animals will be reported subsequently.

Studies are in progress with the collaboration of Dr. John Gofman to determine the chemical nature of opalescence. The opalescence can be eliminated from serum by acetone and ether extraction according to the method described by Blix (*1*). In low speed ultracentrifugation, material that is responsible at least in part for the opalescence rises to the top, to leave a clear infranatant solution.

At present, there is no clear explanation of the mechanism by which radiation produces this opalescence. In view of its apparent relation to death, the phenomenon may not only provide a valuable early measurement of the effect of acute exposure to radiation, but may lead to further knowledge concerning the nature of radiation sickness and its lethal mechanisms.

TABLE 1

DOSAGE OF TOTAL BODY IRRADIATION, APPEARANCE OF SERUM OPALESCENCE, SURVIVAL TIME, LYMPHOCYTE, AND HETEROPHILE COUNTS IN RABBITS

Rabbit No.	Radiation dose	Dose rate	Opalescence* of serum 24 hr after radiation	Days of survival after radiation			Lymphocyte count		Heterophile count	
				Died from radiation	Died other causes	Sacrificed	Before radiation	24 hr after radiation	Before radiation	24 hr after radiation
	r	r/min								
2	200	25.8	None	..	..	28†	4700	1960	4610	2640
1	400	25.8	None	..	..	28†	5560	1030	2570	3180
1	400	23.2	None	..	..	34	2060	840	3230	5810
2	400	23.2	None	..	..	34	1920	990	2880	5346
3	800	47	None	..	..	32	8360	240	2640	7520
7	800	24	Marked	5	..	..	9510	1020	5430	7310
8	800	52	Marked	2	..	..	6980	660	1870	10120
10	800	52	Slight	..	16‡	..	6770	520	1850	9460
11	800	52	Slight	..	..	31	8450	970	1180	9400
12	800	54	None	..	9§	..	2840	150	2440	4980
13	800	54	None	..	..	31	5100	470	2100	3430
5	1000	24	Marked	5	..	..	5740	250	7760	8050
9	1000	52	None	..	..	30	4710	580	6330	6570
14	1000	43.5	None	..	10‖	..	4820	330	2190	3690
15	1000	43.5	Marked	31 hr	..	..	8500	4520	1360	2810
16	1000	44	Moderate	..	..	35	11400	310	2810	7330
17	1000	44	....	20 hr	..	..	6530	....	2400	.....

* Serum was never opalescent except in the period 24 hr after radiation.
† Not sacrificed; 400 r given on 28th day after first radiation.
‡ Died 20 min after Nembutal. Post-mortem: small right hemothorax; otherwise negative on gross examination.
§ Died within 1 hr after Nembutal and cardiac puncture. Post-mortem: marked gastric dilation with moderate pyloric hypertrophy; otherwise negative on gross examination.
‖ Died ½ hr after cardiac puncture; marked hemopericardium present; post-mortem otherwise negative; gross examination.

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An Automatic Analyzer for the Study of Speech in Interaction and in Free Association

Marcel Verzeano and Jacob E. Finesinger

Department of Psychiatry, Harvard University Medical School, and *Department of Psychiatry, Massachusetts General Hospital, Boston*

The study of speech (verbal activity) in the interview situation and in free association is being undertaken as a preliminary investigation of the process of psychotherapy. An attempt is being made to determine the relationship between the tempo and distribution of units of speech and the progress of therapy. Further relationships between verbal behavior and other personality characteristics and clinical diagnosis are being investigated. In previous work the verbal and gestural behavior of subjects and patients have been studied by the use of the "interaction chronograph" (*2*). This device records the interaction by having an observer depress a key during periods of activity and release the key during periods of inactivity. In observing an interview two keys were used simultaneously, one to record the activity of each of the participants.

In the close observation of so many fleeting phenomena even the trained observer is faced with a difficult task. The variability of the observer's reaction time, the practice effect, and the effects of fatigue are the major sources of error. The analysis of the record which is done by other workers may be an additional source of error.

In order to obtain more accurate information, an analyzer was designed and built which automatically performs all the operations required for the recording and analysis of verbal activity—thus eliminating the human observer.

The automatic speech analyzer consists of four parts. The first computes the duration of speech and the total number of units of speech (a unit of speech is defined as any period which is separated from the subsequent one by a minimum pause of a certain duration—in these studies, 0.5 sec). The second part of the analyzer classifies the units of speech in terms of their duration in sec, and gives their frequency distribution. The third part records the number of interruptions and classifies them according to which participant in the interview interrupts and which one stops speaking after the interruption. The fourth part is a tape recorder on which the units of speech are recorded. Two complete units, including the four parts described above, are provided for each of the participants in an interview.

The speech of the two persons in the interview is picked up by unidirectional microphones (cardioid type) whose output is fed into speech amplifiers (Fig. 1). The gain of the amplifiers is adjusted so that the imput of the two microphones will not interfere with each other. The output of the amplifiers is rectified and taken through a clipping circuit which maintains the voltage below a certain limiting level. The output of the speech amplifier, after having gone through the clipping circuit, is fed to an adjustable time delay relay[1] (time delay *A* on diagram) which does not drop out unless the pause in speech is at least 0.5 sec.

Thus when the subject begins to speak the relay pulls on, energizes an electromagnetic counter, and starts an electric timer. When the subject stops speaking *and only after the minimum pause mentioned above has elapsed*, the counter completes the counting of one unit and the

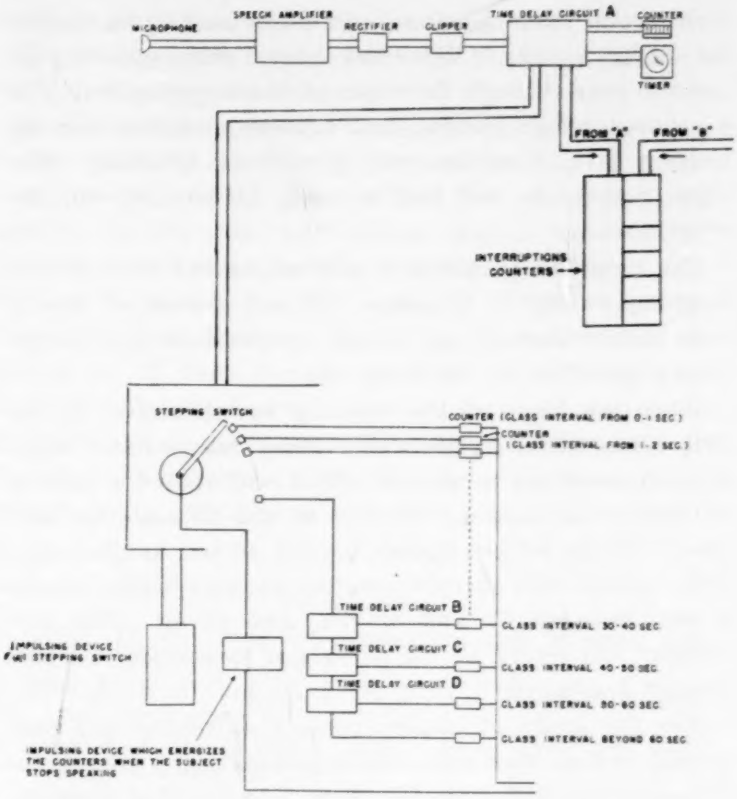

FIG. 1.

timer stops. If the minimum pause does not elapse, the counter does not complete the count and the timer goes on timing whatever follows as one period, until the minimum pause occurs. At the end of the whole interview the counter will show the total number of units of speech and the timer will show the total time in speech.

The total time in silence is obtained by subtracting the total time in speech from the total duration of the interview. The time delay relay also energizes a pen, which leaves a trace of every period of speech on a paper tape, pulled by a synchronous motor, so that each period of speech can be recorded and studied separately, if desired. Two identical channels including microphone, amplifier, rectifier, clipper circuit, time delay relay, counter, and timer are provided; one for the subject, one for the interviewer.

For the study of variability an apparatus was devised which at the end of the interview shows automatically, on a series of counters, the frequency distribution of the

[1] Certain types of time-delay circuits, like the multivibrator ("flip-flop") type, which is used at present in the apparatus described, eliminate the necessity of using rectifiers and clipper circuit. This type of time-delay circuit was designed and built for us by Hanopol and MacLeod, Charlestown, Massachusetts.

durations of the units of speech. (This is the second part of the complete unit.)

This apparatus consists essentially of (1) a timer which provides a short pulse every second; (2) a stepping relay (or stepping switch); (3) a series of time delay relays, (time delay circuits *B*, *C*, and *D* on diagram); (4) a series of electromagnetic counters.

Every time the subject begins to speak the stepping switch begins to move from contact to contact, in steps of 1 sec, under the control of the impulses emanating each second from the timer, and keeps moving as long as the subject speaks. When the subject stops speaking an impulse goes through the wiper of the stepping switch to a counter, which records that unit of speech as one belonging to a class interval of a fixed duration. The wiper returns to zero and is ready to move up for another unit.

For practical purposes it was convenient to limit the stepping switch to 30 steps. If any period of speech lasts longer than 30 sec, it will be classified in a larger class interval in the following way:

When the wiper of the stepping switch arrives at the 30th contact it energizes a time delay relay circuit, which in turn energizes a counter which will record a halt in the subject's speaking between 30 and 40 sec after zero time. If the subject speaks beyond 40 sec, another time delay circuit will energize another counter which records a halt between 40 and 50 sec, and so on. The last counter will record all the periods of speech which lasted beyond 1 min.

For the study of interruptions a switching and computing system was built which automatically counts the interruptions and classifies them into the following six categories:

1.	A begins to speak,	B interrupts,	A stops
2.	A " " "	B "	B stops
3.	A " " "	B "	both stop
4.	B " " "	A "	A stops
5.	B " " "	A "	B stops
6.	B " " "	A "	both stop

One of the great advantages of this analyzer is that it can be used in the study of any phenomenon distributed in time. It can automatically determine the number of occurrences in a given time, the total duration of a series of individual occurrences, and the frequency distribution of the durations of the events studied. From the frequency distribution the standard deviation can be readily calculated.

In our present work we are adapting this apparatus to the study of speech (verbal activity) as it occurs in the interview situation and in free association during psychotherapy. In studying free association we record the patients' verbal productions on a wire recorder and then play the record back into one of the channels of the automatic speech analyzer.

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Increasing the Efficiency of the Laying Hen

H. D. Goodale

Mount Hope Farm, Williamstown, Massachusetts

Work has been under way for many years on increasing the efficiency of the hen by increasing the number and size of eggs, and by reducing losses in the laying houses due to disease. In a long-term attack on this problem and using a unique method of measuring gains, we are able to state that the efficiency of the individual hen, measured by the increase in average weight of eggs per hen per year, has risen by 75.4% since 1923. If the decline in losses is taken into account, the increase in efficiency is 106.8%. This increase has been gained through the consistent use of the family method of breeding—an adaptation of Mendelian principles to the peculiarities of quantitative characters. So far as we are able to learn, no other method of breeding has been developed capable of making so much improvement in efficiency.

References 2–5 give results of the application of this method of breeding in other instances. It has been used by poultry breeders of Massachusetts and vicinity with so much success that poultry breeders in all sections of the United States and Canada are adopting it. The method is effective because each pair of parents is judged by the qualities of all their children. Pairs whose children rate highest in the qualities desired by the breeder are given an opportunity to have more children. Meanwhile, a new generation of parents is selected from members of families with the highest ratings. But of this new generation of parents, only those few whose children rate the highest are retained. This process, repeated generation after generation on a sufficiently large scale, has thus far promoted a constantly rising average of those qualities which the breeder seeks to improve.

The improvement in the efficiency of the laying hen made through consistent use of the family method of breeding suggests that when it is applied to other farm animals and to food plants, similar gains in efficiency will result.

The poultry plant at Mount Hope Farm was begun in 1917. Work on improving the efficiency of the hen began in 1918 with the assembling of the best stock available at that time. Once the assembling of stock was completed, the flock was closed and no other stock added. As it was not necessary for the work to be self-supporting, or to provide a livelihood for a farm family, the work could proceed without the handicaps that confront many poultry breeders. Thus it has been possible to use a method of measuring gains which most poultry breeders have not found feasible.

This method, established in 1923, consists in setting aside entire families of full sisters, which are all held—good, bad, and indifferent—for 15 months after the first hatch is placed in the laying houses. Beginning in 1927, these families have come from parents hatched the previous season. These parents are, therefore, not progeny-

proved. Records are kept in detail. Management has been maintained as nearly as possible like that in 1923.

TABLE 1

Year*	Average† egg weight in oz	Average number of eggs per hen per year	Average weight in oz of eggs produced by each hen per year
1923	1.85	168	310.8
1929	1.85	220	407.0
1946	2.30	237	545.1‡

* Records begin in the autumn of the year the birds are hatched. 1946 is the last year on which completed records are available.

† Taken during the spring.

‡ The 1948 flock promises to exceed 600 ounces.

The birds are housed in units of 100. The number of pullets in this group has ranged from a low of 300 in some years to a high of 800 in other years. The daughters of parents already proved good are kept in a separate group. Their records are not included in this report.

The average annual egg production of these flocks multiplied by the average egg weight gives the amount of product of each hen. From 1923 to 1929, efforts were concentrated on increasing the number of eggs, the size remaining constant. Then, forced by the demand for larger eggs, efforts were made toward increasing and fixing the size of eggs desired by the trade and toward making such gains in egg number as the inverse correlation between egg size and some of the factors entering into egg number permitted. The results of this work are shown by the averages in Table 1.

As Table 1 shows, the average hen in today's flocks is laying 234.3 oz more eggs than in 1923. This is an increase in efficiency of 75.4%.

Inspection of the table shows that the rate of gain from 1929 to 1946 is about half that from 1923 to 1929. It proved much easier to increase number of eggs, leaving egg size constant, than to combine the desired egg size with high rate of lay and early maturity, but this has finally been accomplished.

The enormous losses in the laying houses from deaths due to disease were not generally recognized when the present method of measuring gains in efficiency was established in 1923. This happened because these losses were obscured by the prevalent practice of culling nonproductive birds. A few years later the New Jersey Agricultural Experiment Station (*1*) stated that losses from death and culling during the year sometimes reached 60%. This was confirmed later by the Ohio Agricultural Experiment Station (*6*). Meanwhile, the losses from deaths in our unculled flocks, under exceptionally high standards of sanitation, were reaching the same amount in some years, thus indicating that culling merely anticipated deaths that would otherwise occur.

TABLE 2

Year	Number of pullets at beginning of year	Number dying during year	% dying
1923	304	138	45.4
1929	399	99	25.0
1946	801	148	18.5

The losses in the laying flocks for the three years of Table 1 are shown in Table 2.

A reduction in mortality increases the efficiency of a poultry plant by permitting it to operate toward maximum capacity. If the plant of 1923 had operated at full capacity for the year, i.e., without deaths, each unit of 100 hens would have produced 31,080 oz of eggs. As the records show that deaths occur at a fairly uniform rate throughout the year, each unit operated with an average of 77 hens for the year, thus producing only 23,932 oz of eggs. In 1946, however, with each unit operating with an average of 90.8 hens, it produced 49,495 oz of eggs—an increase of 25,563 oz, or an increase of 106.8% in the efficiency of the plant over 1923.

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Low Temperature and Survival of Embryonic Tissue

John Ferguson

Department of Physiology and Pharmacology, School of Medicine, Creighton University, Omaha

The effect of low temperature on embryonic tissue has a practical as well as a theoretical interest. Various reports have appeared in the literature on hypothermia in relation to neoplastic tissue (*6, 33, 15*). Preyer (*11*) observed that no further development of the incubating chick occurred after the temperature went below 25° C. But the individual embryonic tissues survive a much lower temperature. Hetherington and Craig (*8*) found that small fragments of chick heart may be stored in Ringer-Tyrode's solution at 0° C for as long as 15 days with little apparent effect on viability. Stone (*14*) concluded that the best temperature for preserving alive the enucleated eyes of the salamander was between 4° C and

6° C. Bucciante (*3*) reported on survival time of various tissues of the 6-to-12-day chick embryo when kept in Ringer's solution at 5° C. He found a wide variation—hepatic cells survived 3 days, while skin survived 21 days. Waterman (*18*) has shown that different tissues of the rabbit embryo and of the chick embryo vary in their sensitivity to refrigeration. He found that 5° C is the most suitable temperature at which to preserve embryonic tissue alive, and that at this temperature brain tissue remains viable for a few days, intestine for many days, and skin up to 3 weeks. He also noted that embryonic tissues survive refrigeration at 0° C for a short time only.

To determine the lowest temperature at which embryonic smooth muscle will survive, the amnion of the chick was used. It is a structure containing smooth muscle but devoid of nervous elements of any description (*7*). When suspended as a muscle strip in oxygenated physiological solution at 41° C, it manifests spontaneous rhythmicity, which may be recorded on a smoked drum (41° C is the approximate temperature of bird's blood). Its motility is increased by acetylcholine, eserine, or barium chloride (*1*). This spontaneous activity or its response to a drug served as a criterion of survival after the structure had been exposed to a low temperature.

Fertile hen's eggs were incubated for 10–17 days. The amnion was removed and placed in a test tube which contained 10–15 cc of either oxygenated Sollmann-Rademaekers' solution (designated S-R solution) or oxygenated cooled expressed almond oil. By placing the tube in a freezing mixture the temperature was lowered to the desired level and held at that point, as closely as was practicable, for 10 min. In some instances, when S-R solution was used, ice formed in the tube. When the structure was thawed out, however, and set up as a strip in S-R solution at 41° C, it exhibited spontaneous activity or reactivity to drugs. Several determinations were made after chilling the S-R solution to various degrees. But when freezing occurred the temperature rose rapidly.

To overcome this difficulty almond oil was used. This oil being moderately unsaturated, iodine number 93–100 (*4*), it remained liquid to about –10° C. Vernon (*17*) found that oil dissolved oxygen more readily than water did. After a muscle had been kept in oil at –2° C for 10 min it would still respond, although it had been frozen. However, the preparations which had been kept in oil at –3° C or lower for 10 min neither developed spontaneous rhythmicity nor reacted to eserine or barium chloride. From experiments in which 12 different preparations were used, it was concluded that smooth muscle of the amnion of the chick irreversibly loses its irritability when kept at a temperature between –2° C and –3° C for 10 min.

Others, experimenting with exsected heart muscle of the frog, found the lethal temperature to be about –3° C (*5*). Ice formation may occur in the muscle; still the muscle survives. It is probable that part of the water in the tissue is in the bound form and as a consequence has properties which differ from those of free water. Newton and Gortner (*10*) found that a portion of the water in expressed plant juice took no part in dissolving cane sugar which was added.

However, freezing of the amnion delayed its recovery in developing a spontaneous rhythmicity. Simonin (*12*) observed the injurious effects of ice formation on subsequent growth of embryonic tissue. The influence of the rate at which tissue is frozen is problematical. It is commonly implied that slow freezing is more injurious to tissue than rapid freezing, as larger ice crystals are associated with the former, and hence more mechanical damage results. However, the results of Breedis and Furth (*2*) indicate that cells survive slow freezing better than rapid freezing. But Thoennes (*16*) found that when small bundles of muscle fibers are immersed in liquid air (–195° C) and rewarmed rapidly they can still respond to electric stimulation.

It is apparent that embryonic tissues survive a much lower temperature than the developing embryo. Cameron (*4*) suggested that the coordinating centers of the central nervous system of the frog fail at a temperature much above that which is injurious to the muscle tissue.

In summary, our results show that: the smooth muscle of the amnion of the chick irreversibly loses its irritability when kept at a temperature between – 2° C and – 3° C for 10 min. Recovery is influenced by the degree of chilling. The greater the chilling, the longer is the time required for recovery. Ice forms in the structure in some instances, yet still it may survive. Part of the water of the tissue may be in the bound form. Isolated nerve-free muscle survives a much lower temperature than the intact embryo has been reported to survive.

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Comments and Communications

Replanting "Discarded" Varieties as a Means of Disease Control

Many soil pathogens accumulate to a high degree in the presence of crops toward which they are virulent. The efficacy of crop rotation as a disease control measure lies in the fact that, in the absence of these crops (i.e., in the presence of nonsusceptible crops), the population of a given pathogen materially decreases. Other pathogens, those to which the alternate crop or crops are susceptible, must as surely increase; but by rotating crops subject to widely different pathogens, effective control is often achieved. Why not then, apply the same general practice in control of such pathogens as the grain rusts, *Helminthosporium* of oats, etc.? Such a practice would involve a rotation of host varieties, rather than of distinct, often widely divergent, crop species.

Development of varieties of crop plants resistant to infectious parasites and insect pests is a significant approach to control of plant diseases. There is now widespread recognition on the part of pathologists, breeders, and practical growers that, contrary to earlier opinion, a new variety represents but a temporary advantage.

"The case of Ceres wheat . . . illustrates this point. This rust-resistant variety was distributed in 1926, and by 1934 was grown on more than four million acres. Race 56 of *P. graminis tritici* was first identified in 1928; in 1930 it comprised only 0.2 percent of the wheat stem-rust population . . . increased to 1.0 percent of the population in 1931, to 2.1 percent in 1932, 3.7 percent in 1933, then increased rapidly to make up 33 percent of the population in 1934 and 66 percent in 1938. Ceres was very susceptible to this race, and in the severe stem-rust epidemics of 1935 and 1937, in which race 56 predominated, Ceres was so heavily damaged that it ceased to be generally cultivated." (CHRISTENSEN, C. M. *et al. Ann. Rev. Microbiol.*, 1948, 1, 61.)

Again, recent experience with a group of new oat varieties is significant. These varieties, deriving a highly valued resistance to virtually all races of crown rust from the variety Victoria were planted in this country about 1942. Very soon, reports of a new *Helminthosporium* disease appeared. Now widely recognized under the name *H. victoriae*, this pathogen has eliminated all Victoria derivatives from general use.

While focusing our attention on the striking and often disturbingly rapid increase in "new" races or species of pathogens in the presence of newly emphasized host varieties, we should not forget that some, at least, of the "old" races are correspondingly decreasing. There is likely as significant a decrease in the inoculum of hitherto prevalent pathogens as there is increase in hitherto rare ones! This, coupled with the very possible fact that the old host varieties well may be resistant to the new pathogens, leads to our main thesis: that varietal rotation should be studied as a means of disease control.

The simple fact that a pathogen is new stands as direct evidence that the older varieties were highly resistant to it, and that it was therefore formerly rare. After five or ten years of widespread plantings of a new host type, it may well be that formerly well-known species or races of pathogens will have become scarce, and that older host varieties can be replanted with profit. That many of these discarded varieties are highly desirable is clearly shown by Quisenberry's statement regarding Vicland oats: "It has been stated that in Wisconsin the years 1942 and 1945 were quite similar so far as climatic conditions for oat production were concerned. In 1942 the average yield in the state was 43.0 bushels per acre when only 90,000 acres of Vicland were grown. By 1945 Vicland had increased to nearly 3 million acres and the state average was 51.0 bushels per acre." (QUISENBERRY, K. S. *Chron. Bot.*, 1948, 11, 237.) By selecting for a given crop, such as wheat or oats, several commercially desirable varieties of widely differing susceptibility, it should be possible to work out a type of rotation which would hold disease losses at a low level.

Rotations involving varieties of the same host species will require great care. Yet the principles governing the biological equilibrium between host and pathogen are the same; and such a program seems hardly as difficult as the present constant seach for ever new host varieties. And as McCall points out, "a change in variety requires no change in farm organization, management, utilization, or general plan of financing." (MCCALL, M. A. *Chron. Bot.*, 1948, 11, 273.)

RUSSELL B. STEVENS

University of Tennessee

Age of Canada's Principal Gold-producing Belt[1]

The east-west trending belt of infolded rocks that extends across Ontario into Quebec and includes many of Canada's largest gold mines has long been known to be very old in terms of earth history. The belt is a trough-like strip of volcanic and sedimentary rocks of so-called Keewatin and Temiskaming age. It probably represents the roots of an ancient mountain belt that seems, by crosscutting relationships, to be older than all other similar zones in the Canadian shield area (WILSON, J. TUZO. *Trans. Amer. geophys. Union*, 29, 691).

In southeastern Manitoba there is an area in which granitic rocks have invaded similar volcanics and sediments known locally as the Rice Lake series. These granites have been established by both Pb/U and Sr/Rb measurements (HOLMES, ARTHUR. Rep. Comm. Meas. of Geol. Time, National Research Council 1946–47, p. 39; and AHRENS, L. H. *Nature*, Lond., 1947, 180, 874, to be about 2,000 million years old, the greatest age measured heretofore. This area lies on the line of the above-mentioned east-west belt if extended westward.

[1] Supported by the Office of Naval Research, in the laboratory established by the Geological Society of America.

Work on the helium method of age determination (HURLEY, P. M. and GOODMAN, C. *Bull. geol. Soc. Amer.*, **54**, 305) has continued since the war, with a further understanding of what conditions are necessary for satisfactory age measurements. Measurements on magnetite in certain ''soft host'' environments seem to be on a fairly firm footing.

Only three samples of magnetite from this eastern mineral belt have been measured so far, but the agreement in age with each other, and with the Manitoba area to the west, offers some assurance that the determinations are reasonably correct. Samples 1 and 2 were specimens of magnetite from the Larder Lake district, Ontario. Sample 3 was magnetite separated from a quartz veinlet in a piece of massive sulphide ore from the Horne Mine, Noranda, Ontario.

Sample	Alphas/hr/cm² from thick source*	Helium 10^{-5} cc/g	Age in millions of years†
1	.36	16.9	2100
2	0.15	8.4	2400
3	.26	11.4	2000

* NOGAMI, H. H. and HURLEY, P. M. *Trans. Am. geophys. Union*, 1948, **29**, 335.

† Assuming 90% of radioactivity due to uranium and 10% to thorium, as is about usual in this type of material.

If these ages are correct, they give us our oldest known orogenic belt.

PATRICK M. HURLEY
Massachusetts Institute of Technology

Some Simple Calculations Concerning the Efficiency of the Photosynthetic Mechanism

As is well known, the free energy increase resulting from the photosynthetic reaction

$$CO_2 + H_2O = HCHO + O_2$$

is about 120 kcal. Actually, the total amount of energy absorbed is such that the conversion of light into chemical energy is accomplished with an efficiency of 30% or more (RABINOWITCH, E. L. *Photosynthesis and related processes.* New York: Interscience, 1945. p. 50.) The reaction above consists of the following oxidation and reduction steps:

$$2H_2O = O_2 + 4H^+ + 4e^-$$

$$CO_2 + 4H^+ + 4e^- = HCHO + H_2O$$

In neutral solution the reversible potential of the first reaction is (LATIMER, W. M. *The oxidation states of the elements and their potentials in aqueous solution.* New York: Prentice-Hall, 1938.) +0.81 v with respect to the standard hydrogen electrode while that of the second is −0.49 v, the emf of the total reaction being 1.30 v. One will verify that $1.30 \times 4 \times 23 = 120$ kcal.

The formation of oxygen by oxidation of water is, in general, subject to a considerable anodic overvoltage or activation energy. If in particular the formation of molecular oxygen were to require the prior liberation of atomic oxygen from each molecule of water the minimum anodic potential would be the reversible potential for the reaction

$$H_2O = O + 2H^+ + 2e^-$$

which in neutral solution is +2.00 v.

Similarly the reduction of carbon dioxide to a formaldehyde unit HCHO would be expected to be subject to a considerable cathodic overvoltage. As a matter of fact we have observed (VAN RYSSELBERGHE, P. *et al.* *J. Amer. chem. Soc.*, 1944, **66**, 1801; 1946, **68**, 2047; 2050) that the polarographic reduction of carbon dioxide cannot start until a cathodic potential of about 2 v with respect to the standard hydrogen electrode is reached. The total potential difference would thus be 4 v and the corresponding free energy of the 4-electron oxidation-reduction would be $4 \times 4 \times 23 = 368$ kcal. If the equivalent amount of light energy is absorbed to produce chemical synthesis to the extent of 120 kcal the efficiency of the process is $120/368 = 33\%$, in good agreement with the value given by Rabinowitch (*op. cit.*).

If we accept a mechanism involving eight quanta (which assumption, supported by other evidence, seems logical in view of the fact that there are altogether eight steps of yielding and accepting of electrons for the overall reaction) the average molar quantum would be $368/8 = 46$ kcal, corresponding to an average wavelength of 6210 A. It is interesting to note that the maxima of red absorption in ether solution correspond to 43.3 kcal for chlorophyll a and 44.5 for chlorophyll b (HARRIS, D. G., and ZSCHEILE, F. P. *Bot. Gaz.*, 1943, **104**, 515. See also our polarographic work on chlorophyll (VAN RYSSELBERGHE, P. *et al.* *J. Amer. chem. Soc.*, 1947, 69, 809).

The original free energy level of one mole of CO_2 and two moles of H_2O is −208 kcal. The absorption of 368 kcal brings it up to +160 kcal. If one subtracts from this the loss of free energy of 110 kcal corresponding to the formation of molecular O_2 from the atoms, one arrives at the value of +50 kcal for the free energy of formation of the activated complex CH_4O_2 from the elements in their standard states. This value might lead to useful speculations concerning the structure and properties of this complex. Since the final free energy level of one mole of HCHO and one mole of H_2O is −88 kcal the reaction

$$CH_4O_2 = HCHO + H_2O$$

from the reaction complex to the final products involves a free energy decrease of $50 - (-88) = 138$ kcal.

These considerations are submitted as an extension of the reversible electrochemical point of view already familiar in the field of photosynthesis to the more realistic one of overvoltage and activation. It is hoped that this may be of some use towards the final elucidation of the mechanism of the most important of all chemical reactions occurring in nature.

PIERRE VAN RYSSELBERGHE
University of Oregon

In Memoriam

Paul Rode

1901–1948

Paul Rode, 47, postwar chief of Biological Museums in the French National Service and inspector general of the French Provincial Biological Museums, died on September 23 in Paris. He was the most productive mammalogist in France and the leading candidate for the professorship of zoology in the Paris Museum from which Edouard Bourdelle retired last year on account of age. The collaboration between Paul Rode and the eminent Paris surgeon, Robert Didier, was fortunate indeed for mammalogy. They were two of the foursome (Bourdelle and Bressou were the others) which inaugurated and carried into its 12th (1948) annual volume the French journal of mammalogy, ***Mammalia***. Paul Rode pulled the heaviest load of anyone in that team, as he did in most undertakings in which he participated—never because he was aggressive but because his associates requested him to do so. His loyalty to them and to his science, along with his marvelous efficiency and great industry, were outstanding characteristics. Since he never took his responsibilities lightly, these admirable traits were his undoing, inasmuch as he literally wore out his heart in doing so long the work normal for several men. He is survived by his wife, Renée.

E. Raymond Hall

University of Kansas, Lawrence

William John Dann

1904–1948

William John Dann, professor of nutrition in the Duke University School of Medicine, died at his home in Durham, North Carolina on December 15, 1948, after a prolonged illness. Born in Bath, England on November 9, 1904, he received his formal training in the schools of Sheffield University. In 1925 he began graduate training in the Department of Biochemistry at Cambridge University which led to a Ph.D. in 1930. During these formative years, under the influence particularly of F. G. Hopkins, J. B. S. Haldane, and L. J. Harris, his interest in nutrition developed and thereafter his efforts were concentrated in this field. After receiving the doctorate, he remained in the Nutrition Laboratory at Cambridge for four years as Medical Research Scholar of the Worshipful Council of Grocers and as holder of the Beit Memorial Research Fellowship. During these years he published numbers of papers on the estimation of carotene and vitamin A, and on their metabolism.

In 1934 Dr. Dann came to Durham as assistant professor of physiology and nutrition and assumed responsibility for the teaching of nutrition in the Medical School, becoming professor of nutrition in 1946. Here his interest in vitamin A continued, and with his colleagues and graduate students he developed the first successful colorimetric assay for vitamin A and carotene. He continued to study the physiology of vitamin A deficiency in animals as well as man.

Since Durham was in the area within which pellagra was endemic at that time it was inevitable that he should become interested in the nutritional aspects of pellagra. After establishing that what had been described as "rat pellagra" was in truth due to a deficiency of vitamin B_6 and not of the PP factor, he collaborated with the late Y. Subba Row, then at Harvard, in a search for the actual PP principle. This search culminated in the isolation of nicotinic acid, which had not yet been biologically assayed when the announcement of the significance of this compound was made by the Wisconsin workers in 1937. Most of his scientific efforts in the years thereafter were devoted to a study of the physiological sequelae of niacin deficiency. In all, 58 scientific papers remain as his memorial, as well as vol. XII of the *Biological Symposia*, which he edited as his last scientific endeavor. He had also served on the editorial board of the *Journal of Nutrition* and of *Nutrition Reviews*.

Jack Dann carried over the objectivity of his scientific life to his thinking on all other matters. Remarkably free of preformed prejudices of any sort, he was in all matters a true liberal, forming his own opinion after mature, critical consideration of the subject at hand, yet ever willing to reconsider in the light of new facts.

While a student at Cambridge, he married Eileen Morley, who shared with him his chief interests away from the laboratory—namely, people, books, nature, and their four children. His loss is mourned not only by his family, his friends, and Duke University, but by the scientific community at large.

Philip Handler

Duke University

Book Reviews

Surface active agents: their chemistry and technology. Anthony M. Schwartz and James W. Perry. New York: Interscience, 1949. Pp. xi + 579. (Illustrated.) $10.00.

This is a book that has long been needed. It deals with a modern field of chemistry that has become big business, with applications in many branches of technology. Not only so, but there has developed a whole field of theoretical relations and interesting phenomena that has greatly extended our knowledge of colloids and surface behavior.

The book, after a brief introduction which classifies the materials to be discussed, consists of three parts: Part I devotes 216 pages to processes for synthesizing and manufacturing surface active agents, such as the carboxy acids, the sulfonic esters, the alkane sulfonates, the alkyl aromatic sulfonates, and other hydrophilic compounds, as well as cationic surface active agents of various kinds, and nonionic and ampholytic agents, with brief reference to nonaqueous systems, builders, and mixtures.

This very substantial, comprehensive, and clearly written account consists almost entirely of information published in connection with patents. It is surprising how much of modern organic chemistry has been patented. On every page of this part of the book there are numerous references to relevant patents, amongst which the journal references are completely submerged. The reviewer had not realized what a large proportion of chemical advances have been described in patents rather than in the usual chemical periodicals.

Part II of this very useful book devotes 150 pages to the physical chemistry of surface active agents in theory and practice. It is the most complete discussion of this subject so far available. Not only does it discuss surface and interfacial properties and their relations to chemical constitution, but it gives a careful though somewhat brief review of the properties of solutions of these colloidal electrolytes.

This is followed by a discussion of the "gross effects and technical evaluation of surface active agents," which includes such manifestations as wetting, rewetting, dispersion, foaming, emulsification, and various factors of detergency. Here, as in all parts of the book, the authors have made an attempt to include a complete enumeration of all the significant effects, together with references to the best accounts of specific phenomena.

Lastly, Part III, in another 128 pages, describes the chief practical applications of these important materials throughout the textile industries, in cosmetics and detergents, in pharmaceutical, germicidal, fungicidal, and disinfectant use, in metal technology, in paints and lacquers, inks and pigments, in leather technology, in petroleum and lubricants, in flotation, and in many other branches of industry, such as foods, rubber, and resins. This is a remarkably complete review.

It is evident from the mere enumeration of all these topics, which are presented in well-integrated form, that this book should find a place in every chemistry library. It will be a stimulus to students to look through its pages and see how chemical and physical chemical properties can be applied. It is also an invaluable reference book for those having anything to do with colloidal electrolytes and surface active agents. The authors have rendered a distinct service to chemistry and have added to the value of the mass of information with which they deal by supplying a detailed subject index in addition to a complete author index.

JAMES W. MCBAIN

Stanford University

Temperature and human life. C.-E. A. Winslow and L. P. Herrington. Princeton, N. J.: Princeton Univ. Press, 1949. Pp. xiv + 272. (Illustrated.) $3.50.

This small, well-put-together volume constitutes an excellent handbook for the air conditioning engineer, covering as it does man's *immediate* thermal adjustment to his environment. As a monographic presentation of the authors' fifteen years of work in this field, it is a contribution of value to those engaged in the problems of air conditioning and the physics of body heat loss. By no means, however, does it live up to the broad connotations of its title.

The most important contributions of this Yale group (here presented *in toto*) have been concerned with "partitional calorimetry"—a term they coined to cover their studies on the partitioning of body heat loss between radiational, conductional, and evaporational channels under diverse environmental conditions. They stressed the importance of wall temperature in air conditioning calculations and in other ways provided added refinements for the engineers' activities in this field. However, they consistently refused to accept anything more than a short term relationship between man's thermal environmental and his heat production or general biology. For them, man's slower hormonal response to changes in thermal environment simply does not exist, in spite of adequately documented evidence on the subject.

Your reviewer greatly appreciated being asked to prepare a review of *Temperature and human life*—this field had held his major interest for the past two decades. The book proved rather disappointing, however, in that it has only one chapter dealing—very inadequately—with the more general relationship of climate and the seasons to human biology. The inclusion of only one scientific work published since 1938, among some 39 listed references, clearly dates the authors' thinking in this final chapter. None of the illustrations was prepared later than 1926!

CLARENCE A. MILLS

Laboratory for Experimental Medicine, University of Cincinnati

NEWS and Notes

J. W. Buchanan has resigned as Morrison Professor and chairman of the Department of Zoology at Northwestern University to become Hancock Professor of Zoology and director of research in the Hancock Foundation of the University of Southern California.

Katherine Way, physicist at the Oak Ridge National Laboratory, has joined the staff of the National Bureau of Standards, where she will conduct research in nuclear physics for the Radioactivity Laboratory. Dr. Way will head a project for compiling a table of present-day nuclear data, to which periodic supplements will be added. The table will be available to scientific institutions, universities, the government, industry, and the public.

V. I. Komarewsky, professor of chemical engineering and director of the Catalysis Laboratory at Illinois Institute of Technology, will speak before the Danish Chemical Society in Copenhagen in September. Dr. Komarewsky, who has been a staff member of the Kaiser Wilhelm Institute of Biochemistry in Berlin and the Moscow Academy of Mines, will discuss the catalytic reaction of hydrocarbons.

Merritt Lyndon Fernald, Fisher Professor of Natural History, emeritus, and former director of the Gray Herbarium of Harvard University, has been elected an honorary member of the Societas pro Fauna et Flora of Finland.

Frederick V. Rand, plant pathologist in the Office of Experiment Stations of the U. S. Department of Agriculture, retired on June 30, after 38 years of government service. Dr. Rand did extensive research in the causes and prevention of bacterial wilt of cucumbers, cantaloupes, squashes, and corn by beetle carriers. He served as executive secretary of the division of Biology and Agriculture of the National Research Council and associate editor of both *Botanical Abstracts* and *Biological Abstracts.*

E. W. Comings, professor of chemical engineering at the University of Illinois, will spend a month at the Army Chemical Center this summer. During this time he will make a study of special problems of the Technical Command and the Medical Division. In addition, he will give a series of lectures on fluid dynamics to a selected group of chemical and mechanical engineers of the Army Chemical Center.

Visitors to U. S.

Recent visitors at the National Bureau of Standards were **S. C. Stokes,** British Rubber Producers' Research Association, Welwyn Garden City, Hertfordshire, England; **G. C. Ellis,** metallurgist with the Armament Research Establishment, London; **Goeffrey G. Haselden,** lecturer in low temperature technology, Imperial College, London; **O. H. Saunders,** professor of engineering at London University; **G. P. Douglas** and **E. G. Broadbent,** both of the Royal Aircraft Establishment, Farnborough, England; **Karl G. Ekblad,** civil engineer at Chalmers University, Gothenburg, Sweden, who is here on a year's Scandinavian-American fellowship; **R. H. Field,** chief of the Metrology Section of the Canadian National Research Council; **Roger Coutant,** director general of the Ateliers Pingris et Mollet Fontaine Reimis, Lille, France; **J. Kampe de Fériet** of the University of Lille, France; **L. P. Buseth,** chemical engineer with the A. S. De Forenede Ullvarefabrikker of Oslo, Norway; **Giuseppe Francini,** professor of electrical engineering, University of Bologna; **A. R. Burgess,** of the Imperial Chemical Industries, Ltd., England. Visitors from India were **S. Sankaralingam,** assistant engineer with the government of Madras and at present associated with the University of Louisville; **R. R. Umarji,** professor of mathematics at Gujarat College, Ahmedabad, and member of the Bombay Education Service; and **K. Rajagopalaswami,** chief geologist of the Associated Cement Companies, Ltd., Bombay.

Luis Richard, tuberculosis specialist at the Hospital del Salvador, Santiago, Chile, is working with the Massachusetts State Department of Health in Boston.

H. O. Hartley, Department of Applied Statistics of the University of London, is here for six months to edit "An Index of Tables for Statisticians" on behalf of the Subcommittee on Statistical Tables, National Research Council.

Luis J. Giove Deacon, associate professor of human anatomy at the Medical School of the University of San Marcos, Lima, Peru, is here as consultant on plans for a maternity clinic to be operated by the Maternity Home in Lima, of which he is the director.

Vincent Russo, Buenos Aires economist, is visiting in Washington and New York until August 5, when he will leave for an assignment in Rome, Italy.

Grants and Awards

The National Cancer Institute has made one new grant and 39 renewals totaling \$872,477 to medical schools and one new grant and 15 renewals totaling \$81,439 to dental schools for continued cancer training programs. The two schools receiving aid for the first time are the Chicago Medical School and the University of Nebraska College of Dentistry. Thirty-six special cancer control grants totaling \$550,802 were made to state and local health agencies, universities, hospitals, and other nonprofit organizations.

Henry P. Hansen of Oregon State College was the recent recipient of the 1948 **George Mercer Award** for for the outstanding paper in the field of ecology published in the United States and Canada in 1947. Dr. Hansen's paper, "Postglacial Forest Succession, Climate, and Chronology in the Pacific Northwest," appeared in the *Transactions*

of the American Philosophical Society. Presentation of the Mercer Award, which honors Lieutenant George Mercer, a young British ecologist killed in World War I, was made at the summer meeting of the Western Section of the Ecological Society of America held in Vancouver, B. C., June 14–18.

Meetings and Elections

The Biological Photographic Association, Inc. will hold its 19th annual meeting in Cleveland, Ohio on September 7–10 at the Hotel Cleveland. A program covering all phases of biological photography is being assembled. Inquiries regarding commercial exhibits should be addressed to Fred S. Beal, St. Luke's hospital, 11311 Shaker Boulevard, Cleveland 4. The pre-convention issue of the *B. P. A. Journal* will give complete details of the convention plans.

The Ohio Academy of Science elected as officers for the year 1949–50 at its 58th annual meeting: Paul B. Sears, Oberlin College, president; Rush Elliott, Ohio University, secretary; R. M. Geist, Capital University, treasurer.

At the recent business meeting of the **Paleontological Society of Washington,** held at the U. S. National Museum, the following officers were elected to serve for the year 1949–50: president, James Steele Williams, U. S. Geological Survey; vice president, Helen M. Duncan, U. S. Geological Survey; secretary, David Nicol, U. S. National Museum; treasurer, A. L. Bowsher, U. S. National Museum; and member of Executive Committee, A. R. Loeblich, Jr.

The Illinois State Academy of Science elected the following officers for 1949–50 at its 42nd annual meeting: Thorne Deuel, director of the Illinois State Museum, Springfield, president; Percival Robertson, Principia College, Elsah, first vice president; F. M. Fryxell, Augustana College, Rock Island, second vice president; Leland Shanor, University of Illinois, Urbana, secretary; W. W. Grimm, Bradley University, Peoria, treasurer; and Dorothy E. Rose, Illinois State Geological Survey, Urbana, editor. Leland Shanor is representative to the AAAS.

The Illuminating Engineering Society elected the following new officers as of October 1: Charles H. Goddard, of the Sylvania Electric Products Inc., Ipswich, Massachusetts; S. G. Hibben, Westinghouse Electric Corporation, vice president; E. M. Strong, Cornell University, treasurer; A. H. Manwaring, Philadelphia Electrical & Manufacturing Company, general secretary.

A Wood Symposium, cosponsored by the National Research Council and the Office of Naval Research, was held on June 16–17, at the National Academy of Sciences in Washington, D. C. The conference was called to acquaint scientists and men in the lumber industry with the military requirements of wood and wood products and also to discuss present-day investigations and improvements in the field.

Karl T. Compton, chairman of the Research and Development Board, National Military Establishment, stated in the opening address that "from the standpoint of research and development of the National Military Establishment there is, of course, a responsibility and interest in seeing that anything that can be done to improve materials or equipment for military purposes is done, within the limits of man power and funds available for the work."

The keynote of the conference was conservation. Methods of controlling marine borers are being studied in several laboratories. Physiological studies of wood-rotting fungi, with a view to understanding the basic functioning of the organism in order to find a point at which it might be attacked, are under way at the Department of Plant Sciences at Syracuse University, supported by the Office of Naval Research. Two other ONR-sponsored projects, one at Fordham University and one at the University of Maryland, are exploring the chemistry of lignin. An investigation of the effects of insects on wood is a combined project of the Corps of Engineers and the Department of Agriculture. The chemical utilization of wood and wood residues is being studied at the U. S. Forest Service's Forest Products Laboratory. The Yale University School of Forestry is surveying wood supply and analyzing tropical woods.

Papers presented by Army and Navy participants revealed the enormous amount of lumber required for shore installations as well as for the thousands of small craft needed in landing operations, rescue work, and coastwise transportation. A review of World War II requirements showed that in 1944 close to three million tons were needed for all installations, afloat and ashore; and for wood-packaging alone it was estimated that half the total harvest was going into containers as of August 1945. While peacetime requirements are much smaller, the services realize that in another conflict they would operate in an economy of scarcity rather than one of plenty such as existed at the beginning of World War II. It may seem strange to the layman that, aside from small boats, wood is used to the extent it is in combat ships, but no adequate substitute for wood has yet been found for weather decks and for flight decks. It is required over the steel plates to keep the temperatures in living spaces more equable, to afford better footing in rough weather, and to facilitate topside access for damage control crews during intense fires when steel may reach extremely high temperatures. Teakwood is considered the ace of all decking woods, and for carriers of the Iowa class a total of 110,000 board feet are needed.

Particular attention was given to the marine borers; the several species of these which attack wood bottoms, piles, and other submerged wooden structures cause an estimated 100 million dollars damage a year in the United States and its possessions. A survey of the distribution of borers in the Pacific islands and on the Atlantic coast

was described at the meeting and advances in protective measures were discussed. The Industrial Test Laboratory at the Philadelphia Naval Shipyard reported that the addition of silica to the glue used in plywood offered excellent protection when plywood was used as sheathing on wooden vessels. While many of the projects were of an empirical nature, the need for more fundamental research, especially in the life history of the borers, was stressed. A project of this type has been undertaken by the University of Miami in an attempt to determine the exact stage of wood penetration and also the physiological nature of the action of creosote and other preservatives on the borers.

Although no new preservatives were reported, search and experimentation continues and methods of application and the life span of accepted preservatives are under constant study. The rapidly diminishing supply of lumber in this country would place this commodity in a critical position in the event of another war, and methods of expanding the use of what is available and extending its life after it is fabricated deserve continued research.

NRC News

The Pacific Science Board has made the assignments and field arrangements for the Scientific Investigations in Micronesia Program, an extension of the 1947–49 Coordinated Investigation of Micronesian Anthropology. This new program is financed by the Office of Naval Research in cooperation with the Pacific Science Board and institutions with which the participants are associated.

Nine scientists have been selected and a number have already left for the field. *I. Dyen,* associate professor of Malayan Languages of Yale University, will continue the linguistic work that he started under the CIMA Program on the island of Yap. Dr. Dyen will also spend a short time in Ponape, where he will assist in clarifying certain problems involved in the new orthography evolved from Paul L. Garvin's linguistic work there under the CIMA Program. It is expected that this new orthography will be adopted for general use. *Ann Meredith,* of Radcliffe College, who has been studying social anthropology at Harvard University, expects to study the relationship between the sociocultural system and the socialization process among the native peoples in the Truk area. *Sidney Glassman,* of the University of Oklahoma, will conduct a botanical survey of the island of Ponape with a view to preparing a flora of Ponape. *Irwin Lane,* of the University of Hawaii, will make a botanical survey in the Palaus during a four-month period in which he will study in particular the orchids of that area. He expects to base his operations at the Pacific War Memorial Field Station in Koror, which is being operated by Peter J. R. Hill, the resident naturalist for the station. *F. R. Fosberg,* research associate in the Department of Biology of Catholic University, will make a four-to-five-month study of plant ecology in the Marianas, with special emphasis on environmental factors affecting the distribution of island species. Dr. Fosberg will be engaged in research for the Quartermaster Corps of the Department of the Army. *Donald Anderson,* of Honolulu, Hawaii, will accompany Dr. Fosberg as a botanical assistant. *M. W. de Laubenfels,* professor of zoology of the University of Hawaii, will conduct a sponge survey, during a period of four months, of certain island areas of the Marshalls, and particularly of Ailinglapalap, as well as of the Truk and Palau areas of the Caroline Islands. *Eugenie Clark,* of the American Museum of Natural History, will conduct a four-month research project on the taxonomy of plectognath fishes in the Palaus. She will base her operations at the Field Station of the Pacific War Memorial in Koror. *Robert K. Enders,* professor of zoology at Swarthmore College, will spend a period of four months on the island of Saipan and other islands of the Trust Territory, during which he will carry out ecological studies with special reference to introduced races of rats. Dr. Enders will also conduct a general ecological survey to determine the best locality for future biological research on specific problems.

In addition to this special program, the Pacific Science Board has sent two scientific investigators, supported financially by the Office of Naval Research, into the Trust Territory to conduct further research on a giant African snail, *Achatina fulica.* *Albert R. Mead,* assistant professor of zoology at the University of Arizona, and *Yoshio Kondo,* of the Bernice P. Bishop Museum, will devote close to four months in the Marianas and Palaus to the study of factors which might be helpful in the control of the giant african snail, and will make a survey collection of land shells on selected small islands.

The Food and Nutrition Board of the National Research Council met May 6–7, and heard reports from chairmen of its fifteen committees. The board reaffirmed its position favoring extension of the compulsory enrichment of corn products in those states where corn is a substantial dietary constituent. The board also went on record in favor of rice enrichment, but since rice is not a significant dietary constituent in the U. S. except among isolated population groups, compulsory enrichment was not advocated.

At the request of the U. S. Public Health Service, the board stated its position with regard to the addition of vitamins A and D to skim milk and the use of ascorbic acid in milk for prevention of oxidative off-flavor. The board has continued to adhere to its policy of recommending additions of vitamins to foods only when there is a distinct public health benefit to be attained. The board has no objection to additions of vitamins to milk in reasonable amounts for special purposes, provided the practice encourages consumption of milk and does not increase the cost or reduce the availability of milk or its products to those who need it most.

The board acted to place on a standing basis its *ad hoc* Committee on Food Protection, which has been

reviewing the public health implications of pesticide residues and chemicals being added to foods for special purposes. H. E. Longenecker, dean of the Graduate School, University of Pittsburgh, is chairman of this committee.

New members of the board, nominated for appointment by the NRC under a rotational plan are: W. J. Darby, Vanderbilt University; N. B. Guerrant, Pennsylvania State College; D. B. Hand, New York Agricultural Experiment Station; Ancel Keys, University of Minnesota; O. H. Lowry, Washington University; and R. J. Williams, University of Texas Medical School. These men replaced C. A. Elvehjem, W. E. Krauss, L. A. Maynard, W. H. Sebrell, H. C. Sherman, F. F. Tisdall, and R. R. Williams. Frank G. Boudreau, executive director of the Milbank Memorial Fund, was reappointed chairman of the board for the coming year.

The NRC Committee on Public Health Aspects of Brucellosis met in Washington, May 9–10, with Wesley W. Spink from the University of Minnesota presiding.

The objectives of this committee are: to prepare definitive statements on the problem of brucellosis in animals and its control; to define diagnostic criteria for human brucellosis and to make recommendations for its prevention and treatment; and to encourage research on brucellosis.

A report prepared for the committee by L. M. Hutchings, C. K. Mingle, and W. L. Boyd, "Eradication and Control of Brucellosis in Animals," was revised and approved for publication by the committee. It is hoped that this report will aid the efforts of those groups attempting to resolve the problem of brucellosis in domestic animals.

One of the major problems pertaining to human brucellosis is the standardization of diagnostic procedures among various laboratories. A questionnaire, responded to by 45 states, the District of Columbia, and three Canadian provinces, revealed a marked lack of uniformity in carrying out tests. In view of the situation, the committee is preparing a statement on recommended laboratory procedures for the diagnosis of human brucellosis. In this connection, six to ten state laboratories were to be requested to cooperate in carrying out standardized agglutination tests.

The committee is cooperating in the plans for a symposium on brucellosis, to be held in Washington this fall, and also for an Inter-American Congress on Brucellosis, to be held in Washington in the fall of 1950.

Deaths

Ray Lyman Wilbur, 74, president of Stanford University for 27 years and its chancellor since 1943, died June 26. Dr. Wilbur was U. S. Secretary of the Interior under President Hoover and served as president both of the American Academy of Medicine and the American Medical Association.

Birbal Sahni, 58, professor of botany and dean of the Faculty of Science at Lucknow University, India, died April 9. A fellow of the Royal Society, Dr. Sahni had recently been elected to preside over the International Botanical Congress next year.

Irving S. Lowen, 38, associate professor of physics at the Washington Square College of New York University, died on April 23. Dr. Lowen was research associate with the Manhattan Project and an associate of the National Defense Research Committee during the war, and later a consultant of Brookhaven National Laboratory and director of the U. S. Navy project on cosmic rays.

Preston Strong Millar, 69, president of Electrical Testing Laboratories, Inc., died June 17 at his summer home at Glen Spey, New York. Mr. Millar was one of the founders of the Illuminating Engineering Society, its president in 1913, and the recipient of its gold medal in 1945.

The ionosphere is being explored by lightweight rockets, which carry a hundred pounds of recording instruments into the upper atmosphere. In Navy tests at the White Sands Proving Ground in New Mexico the Aerobee rockets reached a velocity of 4,100 feet per second in about 45 seconds, and attained an altitude of over 70 miles. The Aerobee is about 20 feet long and 15 inches in diameter, and weighs 1,665 pounds. It was designed and constructed for the Navy Bureau of Ordnance by the Aerojet Engineering Corporation of Azusa, California, under the technical guidance of the Applied Physics Laboratory of Johns Hopkins University.

Data which the instruments gather in flight are obtained by means of radio transmission from a telemetering set, and by recovery of photographic film from the wreckage. Impact shock in landing has been reduced by explosive separation of the tailfin structure from the rocket during descent and the film, which is contained in heavy metal casing, is recovered intact.

Through study of the ionosphere, scientists hope to answer questions regarding the composition of the atmosphere at high altitudes, cosmic rays, and the sun's spectrum.

The ancient city of Madaba, which served as a diocesan center for Christians from about 300 to 600 A. D., will be the subject of an archaeological survey this summer. A. Henry Detweiler, professor of architecture at Cornell University, left June 26 to make a topographical study of the city, which has not been thoroughly explored since 1901. The site previously yielded the Madaba Map of Palestine—a mosaic on a church floor which showed how the country looked during the Byzantine period. Professor Detweiler, in addition to surveying Madaba, will serve as director of the American School of Oriental Research in Jerusalem during the summer.

The professional library of the late **Burton E. Livingston** has been given to the Hebrew University, Jerusalem. Dr. Livingston was professor of plant physiology and forest ecology and director of the Laboratory of Plant Physiology at Johns Hopkins University, Baltimore.